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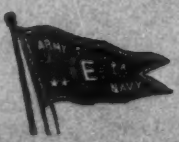

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TRANSPLANTABLE OSTEOGENIC SARCOMAS INDUCED IN RATS BY FEEDING RADIUM *

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Radium poisoning came into prominence as an important industrial hazard with the reports by Hoffman,[1] Castle, Drinker and Drinker[2] and Martland and co-workers[3-6] on the fate of employees in the luminous dial industry in New Jersey. The girls who were employed to paint the figures on clock and watch faces used a paint consisting of zinc sulfide rendered luminous by the addition of 1 part in 40,000 of radium, mesothorium and radiothorium. Prior to the recognition of the danger involved, these workers were in the habit of bringing their brushes to a sharp point by drawing the bristles between their moistened lips, and thus ingested each day a small amount of radioactive material. Deposits of radium within the body may be acquired in other ways[7] but the luminous paint and dial industry, in spite of modern safety measures, still presents the greatest hazard.

Radium is similar in many ways to calcium and to lead and appears to follow the same metabolic pathways within the body.[8-10] When radium is ingested most of it passes through the intestinal tract and is excreted with the feces. The small amounts which are absorbed are at first distributed in the soft tissues but are soon transferred to bone. In human cases of chronic poisoning practically all of the radioactive material is contained in the skeleton.[7] In like fashion, Thomas and Bruner[10] found that 99 per cent of the retained radium in rats was deposited in bone 2 to 8 months after injection. The exact mode of deposition is not known, but it is quite possible that radium substitutes for calcium in the formation of bone salts. The early site of skeletal deposition is in the delicate spicules of trabecular bone, but subsequently a more even distribution between cortical and trabecular bone is attained[8,11] as has been shown for other heavy metals.[12] Deposits of radium in trabecular bone are fairly easily mobilized but when trans-

* Received for publication, February 15, 1943.

ferred to dense cortical bone they are relatively stable, exchange is sluggish, and excretion is minimal.[8] Early in the course of human poisoning excretion may amount to as much as 1 per cent of the total body deposits each day, but with the passage of time it falls to levels as low as 0.005 per cent a day.[8, 10]

The total body content of radioactive material in persons who have died of radium poisoning has ranged from 2 to 180 μg. (micrograms).[7, 13] It seems clear that such small quantities do not act as chemical poisons but as sources of radiant energy. The tissue damage results from the cumulative effects of constant exposure to alpha, beta and gamma radiation. Since alpha particles have little ability to penetrate tissues they are quickly absorbed and exert a concentrated injurious effect in the immediate vicinity of the radium deposits. They are, therefore, more effective in producing local tissue damage than the more penetrating beta and gamma radiations and are chiefly responsible for the ultimate lethal action of minute deposits of radium in the body.

Martland[13] has reported in considerable detail the clinical course of radium poisoning and the pathological findings as seen in a group of dial painters. The principal symptoms reflect damage to the bones and bone marrow, as would be expected from the selective localization of radium in the skeleton. Heavily poisoned persons suffered from leukopenic anemias and necrosis of the bones of the jaw. Others remained in apparent good health for months or years after exposure and then developed chronic, crippling bone lesions or "radiation osteitis." The lesions were widely distributed over the skeleton and were characterized roentgenologically by patchy areas of increased and of decreased bone density. Pathologically the bone marrow in these areas showed immaturity and regenerative hyperplasia with occasional areas of aplasia and fibrosis. The most remarkable feature of Martland's series of cases was that 5 osteogenic sarcomas occurred among 18 persons who died from radium poisoning.

Attempts to reproduce the main features of radium poisoning in laboratory animals have met with considerable success. In general the radium has been introduced by injection rather than by feeding and the doses have often exceeded the range covered in cases of human poisoning. The blood and bone marrow changes seen in human beings have been reproduced and occasional osteogenic sarcomas have been obtained. A number of brief reviews are available on animal experiments[14–16] as well as several on human poisoning and its treatment,[7, 8, 13] but only a few of the more pertinent animal experiments will be cited here.

Thomas and Bruner[10] injected 8 rats subcutaneously with doses of

40 to 60 μg. of radium chloride. The animals retained an average of 25 per cent of the radium and showed progressive deterioration of the peripheral blood picture. At autopsy, 183 to 258 days after the first radium injection, 99 per cent of the retained radium was found in the bone ash. Hyperplastic marrow was present in the mid-shaft of the long bones and aplastic marrow at the ends. The bones were fragile and regions of necrosis were present. The spleen and lymph nodes showed atrophy, hemosiderosis and hematopoiesis. One rat developed motor disturbance of the hind legs shortly before death, but no tumor was discovered in any of the animals.

Sabin, Doan and Forkner[16] poisoned 9 rabbits with repeated intravenous injections of radium or of mesothorium. One rabbit received a total of 70.4 μg. of radium and died with osteogenic sarcoma of the humerus 18 months after the first injection. A second osteogenic sarcoma was found in a rabbit which had received 46.2 μg. of mesothorium over a period of 11 months. Two other rabbits in which no tumors were found developed "marked weakness of the muscles or paralyses" shortly before death.

One of the few animal experiments in which radioactive substances were fed rather than injected was carried out by Rosenthal and Grace.[15] Four rabbits were fed radium sulfate by dropper three times daily for 90 days, to a total dose of 100 μg. per animal. The animals survived 6, 15, 16 and 21 months respectively after the first radium feeding. Abscesses of the jaw, osteoporosis, newly formed subperiosteal bone and spontaneous fractures were observed but no tumors. Terminal paralysis of the hindquarters developed in one animal and was attributed to spontaneous fracture of a vertebra.

Martland's[13] view that the damage suffered by dial painters was due almost entirely to alpha radiation, while beta and gamma radiations were negligible factors, is probably correct. However, no satisfactory evidence has yet been educed that different kinds of ionizing radiation produce qualitatively different biological effects. The observed differences have been quantitative and are explainable on the basis of variations in the intensity and distribution of ionization in different parts of the body. In animal experiments, gamma radiation, as well as mixed beta and gamma radiation, have been shown capable of inducing osteogenic sarcoma.

Ross[17] implanted a platinum tube containing 0.1 mg. of radium under the periosteum of the rib of a rabbit. Two years later the tube was found encased in a large mass of osteogenic sarcoma. The wall of the platinum tube had a thickness of 0.55 mm., which is sufficient to filter off all the alpha radiation and practically all the beta radiation.

Her inference that the tumor resulted from exposure of bone and periosteum to gamma radiation appears to be justified. Schürch and Uehlinger,[18] in a similar experiment, obtained an osteogenic sarcoma of the jawbone of a rabbit 1½ years after the subperiosteal implantation for 20 days of a platinum needle containing 1 mg. of radium. Hellner[19] produced osteogenic sarcoma in the knee of a rabbit by the external application of a platinum-iridium tube containing radium.

Combined beta and gamma radiation was effective in the hands of Lacassagne and Nyka.[20] Osteogenic sarcomas were induced in the region of the sella turcica in 3 of 11 rabbits that survived over 1 year after the implantation of glass radon tubes in the hypophysis. The induction times of the tumors were 14, 13 and 12 months, and the strengths of the radon tubes were 0.69 mc., 1.08 mc. and 0.83 mc. respectively. The alpha particles from radon do not penetrate the wall of an ordinary glass capsule, so that the tissues of these rabbits were exposed to mixed beta and gamma radiation. The total doses used were much smaller than might appear since radon has a half-life of less than 4 days and emits only 133 mc. hrs. (millicurie-hours) of radiation in the total decay of one initial millicurie. The animal exposed to 0.83 mc. thus received a total dose of only 110 mc. hrs. A comparable dose could be delivered by exposing the animal to 1 mg. of radium for 110 hours.

The studies to be reported in this paper concern the fate of rats given radium by mouth, thus duplicating the mode of exposure to which the radium dial painters were subjected.

Materials and Methods

Thirteen male Wistar rats, 5 months old, were each fed a total of 100 μg. of radium chloride. The stock radium solution contained 1500 μg. of radium taken up in 13 cc. of glycerine and 26 cc. of normal saline solution. Each rat was fed 3 drops of this solution daily for a period of 20 days. Detailed measurements of the retention and excretion of radium will be reported in a separate communication.[21] It will suffice to say that about 95 per cent of the radium had been excreted by the tenth day after the final feeding and that an average of 2 μg. remained in the body of each rat at the end of 10 months. All surviving animals, together with 2 controls, were x-rayed on October 31, 1941, 10 months after the radium feeding, and no significant changes in the skeletons were seen. Whenever an animal appeared moribund, it was killed with ether and autopsied.

Representative tissues were fixed in acetic acid-Zenker's solution, stained with eosin and methylene blue, phosphotungstic acid-hema-

toxylin, and Giemsa stain and examined microscopically. Whenever a tumor was found, fragments were transplanted subcutaneously into other rats.

Two of the primary tumors and a number of the transplanted tumors were assayed chemically for alkaline phosphatase activity and several were selectively stained for alkaline phosphatase by the method of Gomori.[22]

Results

None of the rats showed objective signs of disease during the first 8 months of the experiment, but then, one by one, 9 of them developed paralyses of the hindquarters and the others became progressively emaciated and cachectic.

Autopsy Findings

The important features of the autopsies are summarized in Table I. The outstanding observation was that 9 of the 13 rats developed osteogenic sarcomas in time intervals of 253 to 426 days after the first radium feeding. The average induction time of the tumors was 365 days. The primary growths were in the vertebrae in 7 rats and in the pelvic bones in 2. One of the vertebral tumors was cervical, 3 were thoracic and 3 lumbar. All of the growths were so situated that they either had, or might have, exerted pressure on the spinal cord or major nerve roots (Fig. 1), thus explaining the terminal paralyses that occurred in all but 1 of the tumor-bearing rats. However, 1 rat which had no tumor developed paralysis of the hind legs.

Metastases were found at autopsy in 2 of the 9 rats. In 1, the pelvic lymph nodes were invaded from a tumor primary in the ilium. In the other a primary sarcoma of the third thoracic vertebra was associated with a solitary nodule of bone-forming tumor in the lower pole of the spleen. Since the spleen has no afferent lymphatics, the splenic metastasis must have been blood-borne, whereas lymphatic spread was probably responsible for the lymph node metastases.

All of the tumors were so similar in gross and histological appearance that they may be described together. Grossly they were firmly attached to bone and extended as nodular projections into the surrounding soft parts. Although the borders were sharply defined there was no plane of cleavage between muscle and tumor. When there was extension into the spinal canal or thoracic cage, the tumors did not break through the lining membranes but presented as smooth, pale pink, epidural or subpleural masses (Fig. 1). All of the growths were hard centrally but carried a superficial layer of soft, pink, cellular tissue. The consistency was rigid, gritty and granular but never as hard as that of mature bone.

TABLE I

Pathological Changes in Rats Poisoned with Radium

Animal no.	Survival time after feeding radium	Osteogenic sarcoma	Location of primary tumor	Bone marrow changes			Bone changes	
				Hypoplasia	Immaturity	Hemosiderin	Necrosis	New bone
	(*days*)							
25630	253	+	Vertebrae, T. 3, 5, 6	+++	++	++	+	+
25631	318	+	Vertebrae, C. 5, 6, 7	+++	+	+	++	+
25632	398	+	Both ilia	+	++	+	++	++
25633	398	+	Vertebra, L. 1	+	+	+	+	+
25634	306	o		+	+	+	+++	+++
25635	426	+	Vertebrae, T. 1, 2	+	+	++	++	+
25636	289	o		++++	++	++	+	+
25637	388	+	Left ilium	++	+	+	+	+
25638	305	o		+++	++	++	++	+
25640	418	+	Vertebra, L. 2	++	+	+	++	+
25641	263	+	Vertebra, T. 3	++	++	++	+	o
25642	419	+	Vertebra, L. 4	+	o	+	+	+
25643	334	o		+++	+	++	++	++
Controls	Survival time							
25644	334	o		o	o	o	o	o
35647	530	o		o	o	o	o	o

Microscopically the neoplasms showed only minor individual variations (Fig. 2). They were all continuous with normal bone, often showing erosion or destruction of the cortex and growth within as well as outside the marrow cavities. It was impossible to determine in most instances whether they were of periosteal or endosteal origin. The advancing border of the tumor was made up of a compact mass of fusiform, stellate, or oval cells with a little pink, homogeneous, intercellular substance. Mitotic figures were present in considerable numbers and occasional tumor giant cells were seen. On passing from the periphery toward the central portion of the tumors pink-staining intercellular substance began to appear in greater quantity between tumor cells, ill defined at first but soon passing over into well-formed neoplastic bone. In the more mature regions a single layer of tumor cells covered the surface of each spicule of bone and closely resembled a row of normal osteoblasts. Calcium stains showed the central portions of each spicule of newly formed bone to be well calcified while the margins were apparently made up of uncalcified osteoid substance. The deeper portions of some of the growths contained large areas of degeneration with death of all cells but no evidence of hemorrhage or inflammation. No cartilage was present in the tumors. The metastatic nodules, found once in the lymph nodes and once in the spleen, were indistinguishable structurally from the primary growths. Two of the primary tumors were more anaplastic than the other seven and showed greater local invasiveness of muscle and smaller amounts of neoplastic bone formation.

The vertebral bone showed remarkable changes even in those regions uninvolved by tumor. In every animal there were patchy areas of sterile bone necrosis characterized by an absence of living osteocytes in the bone lacunae. The entire thickness of the cortex was often necrotic in addition to many spicules of trabecular bone in the marrow cavity. Although osteoclasts were rarely found, the necrotic bone usually showed irregularly scalloped regions of resorption along its free margins and sometimes the apposition of a surface layer of new living bone of fibrillar structure (Fig. 3). Poorly formed spicules of new bone were present in the vertebrae of all but 1 of the animals (Fig. 4). As a rule they extended longitudinally into the marrow cavity from the bony plate at one end of a vertebra. The osteocytes of the new bone were elongated and the matrix structure was loose and fibrillar. Some areas were acellular, suggesting that necrosis had taken place even in the newly formed bone. The old bone trabeculae in the vertebrae were coarse and irregularly thickened (Fig. 1), particularly in regions of

bone necrosis, as though the normal processes of bone resorption had been impaired.

Similar areas of bone necrosis with peridental suppuration but without new bone formation were found in the mandible or maxilla in all of the 4 experimental animals in which the jaw bones were examined. The peridental suppuration is of doubtful significance since a somewhat similar change was found in 1 of the controls. The explanation may lie in the fact that the animals were fed throughout the experiment on a diet of soft, ground food which furnished little exercise for the teeth and gums.

In addition to the changes in the bone itself, the bone marrow of all the experimental animals showed extensive hypoplasia and atrophy. In the vertebral bodies most of the surviving cells were gathered in the central portion of the marrow cavity while the peripheral portions showed extensive fatty aplasia (Fig. 5). In other parts of the skeleton the process was not so uniform and irregular regions of atrophy or hypoplasia alternated with more cellular regions. In the atrophic regions the marrow was replaced by fat cells together with a few scattered macrophages and stellate mesenchymal cells. Only slight fibrosis was present and very little of the "gelatinous edema" or mucoid degeneration which has been described in radiation damage to bone marrow.[13, 17] More striking were the deposits of hemosiderin in the bone marrow of all of the animals examined. In the cellular portions of the marrow little or no hemosiderin was apparent and the heaviest deposits were present in macrophages between the fat cells of the atrophic regions. The only consistent abnormality of the surviving marrow cells was generalized immaturity. In some animals the proportion of erythroblastic and myeloblastic cells was normal while in others cells of one or the other series predominated. The number of megakaryocytes was reduced in proportion to the other marrow elements. Evidences of active cell destruction were not seen in any of the animals nor were areas of hyperplastic marrow encountered.

The spleen showed changes in all of the animals, fairly uniform in character, but varying in degree. In all cases the number of lymphocytes was reduced, the malpighian bodies were small and the total bulk of the spleen was slightly reduced. No fibrosis was apparent but hemosiderin deposits were abundant, both free and within phagocytic cells. Active centers of erythropoiesis were present in most instances. The lungs, liver, kidneys, testes, spinal cord and intestinal tract showed no constant changes attributable to radiation.

The significant histological changes, apart from the tumors, may be summarized briefly as follows: patchy sterile necrosis of bone with

atypical new bone formation; focal atrophy, hypoplasia, immaturity and hemosiderosis of the bone marrow; atrophy and hemosiderosis of the spleen with focal erythropoiesis.

Tumor Transplants

Attempts to transplant the tumors yielded several interesting results. The experimental animals belonged to the Wistar strain, but at the time of the first autopsies no other Wistar rats were available and the tumors were transplanted to stock rats. All of the transplants failed to grow with the single exception of one in an animal which had been irradiated 3 days previously.* The radiation was given in an attempt to favor the successful growth of an heterologous tumor transplant pursuant to reports of such action by Clemmesen.[23, 24]

The tumor grew slowly for 3½ months, reaching a diameter of 1 cm. Roentgenographs taken at this time showed a heavily calcified nodule. A portion of the tumor was then excised under ether anesthesia and transplanted to 6 young Wistar rats. The original transplant has since remained static and is still present as a hard, subcutaneous nodule, 5 mm. in diameter, 16 months after transplantation. Portions of this tumor transplanted to the original Wistar strain grew progressively and reached diameters of several centimeters in 3 months. At autopsy 2 of these rats had extensive metastases of bone-forming tumor in the lungs. The tumor has now been carried through 7 serial generations of transplants. About 50 per cent "takes" are obtained and palpable nodules appear 1 to 2 months after transplantation. Occasionally a transplant which has apparently failed to take will begin to grow after a latent period of over 6 months. Pregnancy appears to accelerate growth. Once established, the tumors grow slowly and do not kill their hosts for a matter of 3 to 6 months. Death is usually caused by extensive central necrosis and ulceration of the transplant. Throughout a period of over a year of serial transplantation this tumor has retained its original histological characteristics and its ability to form bone.

Two other primary tumors were successfully transplanted to young Wistar rats but neither has been carried beyond the first generation.

Phosphatase Studies

Since a high concentration of alkaline phosphatase has been consistently demonstrated in human osteogenic sarcomas,[25] similar studies were made on the rat tumors. Chemical assays of alkaline phosphatase

* The treatment consisted of 1,000 r. of roentgen rays to a 5 by 5 cm. portal at 200 kv., 8 ma., 20 cm. skin-target distance and 0.25 mm. Cu filter, kindly administered by Dr. Milford Schulz.

activity were made on portions of the original neoplasms from rats 25640 and 25642 as well as on several rats with second, third and fourth generation transplants. The method employed was that of Martland and Robison[26] as modified by Franseen and McLean.[25] The results as summarized in Table II show that the rat sarcomas, like their human counterparts, have a high phosphatase content and that the phosphatase level is maintained in the transplanted tumors. The two sarcomas which were analyzed for calcium and phosphorus contained less of these minerals than is present in mature bone (Table II) but about the same amount as is found in the deer antler during the early stages of calcification.[27]

TABLE II

Alkaline Phosphatase and Mineral Content of Osteogenic Sarcomas in Rats

	Alkaline phosphatase	Calcium	Acid-soluble phosphorus
	Kay units	*mg./gm. of fresh tissue*	*mg./gm. of fresh tissue*
Normal rat bones			
Vertebrae	15–26		
Femur (shaft)	12–13		
Femur (head)	18		
Osteogenic sarcomas			
2 Primary tumors	49.2–88		
3 Second generation transplants	66.3–88.2	29.0	13.9 (74% moisture)
1 Third generation transplant	75.3		
3 Fourth generation transplants	43.7–61.5	32.8	14.5 (53% moisture)

In order to observe the minute distribution of phosphatase in and around the tumors, alkaline phosphatase stains of microscopical sections were made according to the ingenious method of Gomori.[22] Because of the high phosphatase content, all of the osteogenic sarcomas stained very darkly, but the enzyme appeared to be most heavily concentrated in the tumor cell nuclei. The enzyme was confined rather sharply to the neoplasm and showed very little diffusion into adjacent normal tissues (Fig. 6).

Tissue Cultures

Small fragments of two primary osteogenic sarcomas were explanted in tissue culture by Dr. Austin M. Brues. Good growth was obtained in both cases by the third day, but attempts to transplant the cultured fragments back into rats were not successful. Tissue stains failed to show any phosphatase in the new cells which had proliferated about the borders of the explant, although the enzyme was still stainable in the explant itself.

Discussion

The histological changes observed in these 13 rats, following the ingestion of radium, have been described in some detail since they parallel so closely the pathological findings of radium poisoning as seen in the New Jersey dial painters. The outstanding difference has been the uniform finding of hypoplastic bone marrow in the rats, whereas many of the dial painters had a hyperplastic, regenerative marrow.

The difference may be due to the fact that the dose of radium which the rats retained throughout the experiment was approximately 2 μg. per animal and was thus considerably greater in proportion to body weight than in the dial painters whose total body content ranged from 2 to 180 μg.[7, 13] In both the human beings and the rats a prolonged period of apparent good health was followed by skeletal changes and the ultimate development of a high percentage of osteogenic sarcomas. Profound damage to blood-forming organs was found at autopsy, together with widespread necrosis of bone. Many of the osteogenic sarcomas in the rats were found in the vertebral bodies, while in the human beings they were in the pelvis, lower extremity and elsewhere. The difference in distribution may be accidental but might be related to the fact that the epiphyses in the rat remain open throughout life and bone growth continues to a slight extent even in the vertebrae.

The nonmalignant changes produced in bone by heavy irradiation have never been adequately studied either in human subjects or laboratory animals.[14, 28] Roentgenographically, patchy areas of bone rarefaction and condensation may be seen as a result of either radium poisoning or external irradiation.[8,13, 29] The corresponding histological changes have not been clearly defined, perhaps because of a lack of interest among pathologists and perhaps because the bone changes are not readily seen in microscopical preparations. "Radiation osteitis" with its associated rarefaction and spontaneous fractures [8, 13, 29] may result more from damage to the internal structure of bone matrix and disturbances of mineralization than from direct resorption of bone trabeculae. However, the striking microscopical changes in the bones of our rats, reported here only as incidental findings, would suggest that the possibilities of the histological approach have not been exhausted.

Paralysis of the hind legs was a constant clinical feature in all but one of the rats bearing osteogenic sarcomas and was observed in one rat in which careful search revealed no tumor. The latter instance is not explained, but in all of the others the tumor was so situated that it could exert direct mechanical pressure on the spinal cord or nerve roots.

It is interesting that Thomas and Bruner,[10] Sabin, Doan and Forkner [16] and Rosenthal and Grace [15] all described paralyses of the hind legs in some of their radium-poisoned animals in which no tumors were found at autopsy. Since nervous tissue is highly radioresistant, the paralyses were probably secondary to skeletal damage and not a result of direct radiation damage to the brain or spinal cord.

Thomas and Bruner [10] injected 40 to 60 μg. of radium chloride into each of 8 rats and the animals died without tumors 183 to 258 days after the first injection. In our experiments the first osteogenic sarcoma was found 253 days after feeding radium, a time interval corresponding almost exactly to the longest survival of Thomas and Bruner's rats. It is reasonable to assume that these workers would have observed osteogenic sarcomas if their doses of radium had been small enough to permit their animals to survive a little longer. A comparison of our results with theirs brings up another interesting point. Subcutaneous injection of radium in their hands resulted in an average retention of 25 per cent, and the total body content of radium in their animals at death ranged between 8 and 17 μg. Our animals retained only about 2 μg. If their experiment and ours may be considered comparable in other respects, one might conclude that the induction of osteogenic sarcoma by radioactive deposits in the bones of rats requires a time interval of about 8 months and cannot be greatly hastened, even though the amount of radioactivity in the bones be increased by a factor of 5 or more. Such an inference is not inconsistent with what is known of tumor induction by other forms of radiation. A suitable latent period must always elapse, regardless of the dose, before tumors appear. The same principle applies to chemical carcinogenesis, for here, as well, increasing the dose of the carcinogenic agent will not shorten the latent period for tumor induction beyond a fixed minimum.[30] One might speculate on the bearing of this latent period on the mechanism of tumor induction. If the action of the tumor-inducing agent were simple and immediate, acting directly, let us say, to produce atypical mitoses or mutations in exposed cells as suggested by Henshaw,[31] and others, we would expect greater doses to exert a greater effect and by the rules of probability result in the earlier appearance of a tumor. On the other hand, if the agent merely produces a specific type of damage and the neoplasm arises indirectly at some stage in the process of repair, then the minimum latent period might be determined by the rate of evolution of the repair process irrespective of the magnitude of the initial injury. It is difficult to determine the exact origin of the sarcomas in our rats. The new formation of bone in the vertebrae appeared to be a reparative rather than a neoplastic reaction since it was most promi-

nent in the regions of greatest bone damage and since the spicules of new bone were so oriented as to lend support to regions weakened by necrosis. However, Martland [13] believed that "radiation osteitis" is potentially a malignant lesion and the process as seen in rats could readily serve as the starting point for the growth of a malignant bone-forming tumor.

Summary and Conclusions

Osteogenic sarcomas appeared in the vertebrae or pelvic bones in 9 of 13 male Wistar rats after feeding each animal 100 μg. of radium. Within 10 days after feeding, the animals had excreted 95 per cent of the radium and the average amount retained at the end of 10 months was only 2 μg. The induction time from the first radium feeding to the discovery of the tumors was 253 to 426 days and averaged 365 days. Two of the nine primary tumors metastasized, one to the pelvic lymph nodes and one to the spleen. Three of the tumors were successfully transplanted to other rats. One has been maintained through 7 serial generations. The high phosphatase level present in the original tumors has been maintained in subsequent transplants.

The bone marrow of all of the rats was hypoplastic and showed generalized immaturity and abnormal deposits of hemosiderin. Extensive hemosiderosis was present in the spleens together with slight atrophy and extramedullary hematopoiesis. Widespread necrosis of bone was present in all of the rats and atypical new bone formation was found in all but one. Partially frustrated attempts to regenerate bone in regions of radium necrosis may be responsible for the ultimate appearance of osteogenic sarcomas.

In conclusion, the osteogenic sarcomas and other pathological changes consequent to radium poisoning in human beings have been reproduced in rats with a fair degree of fidelity and a readily transplantable osteogenic sarcoma of rats has been obtained.

Assays for alkaline phosphatase activity were conducted by Miss Dorothy M. Tibbetts. Dr. Austin M. Brues explanted fragments of two tumors in tissue culture.

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[*Illustrations follow*]

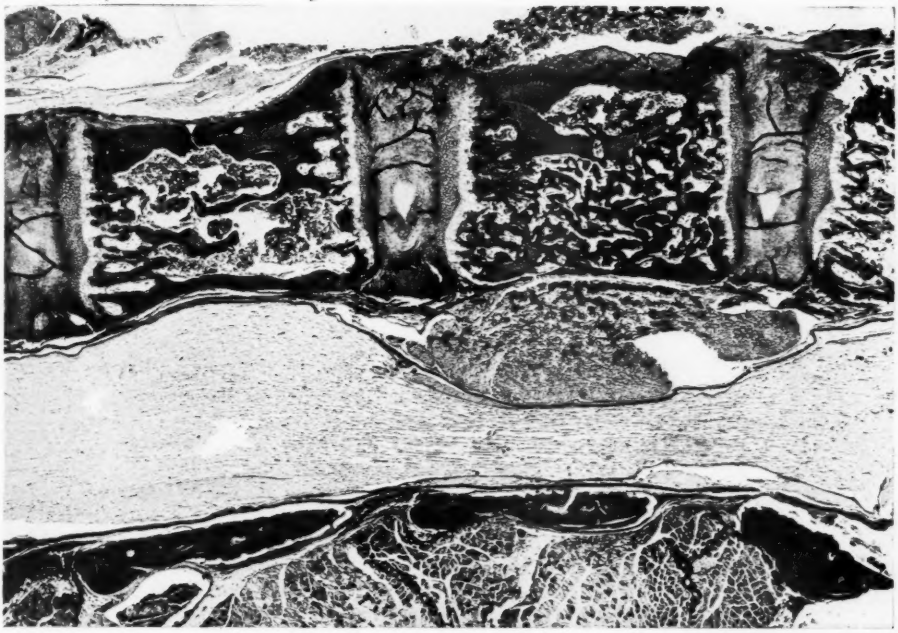
DESCRIPTION OF PLATES

PLATE 1

FIG. 1. Radium rat 25635. Primary osteogenic sarcoma of second thoracic vertebra. The tumor has grown by expansion and has compressed the spinal cord without invading the dura. Bone changes are present in the corresponding vertebral body. × 13.

FIG. 2. Radium rat 25641. Primary osteogenic sarcoma. × 235.

1



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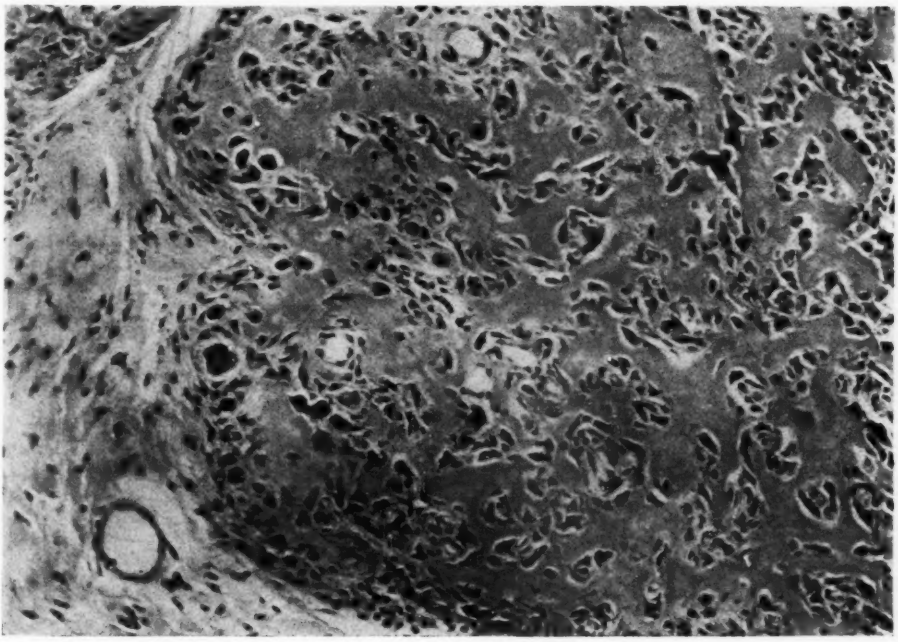

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PLATE 2

FIG. 3. Radium rat 25634. Periosteal new bone formation overlying area of bone necrosis in the cortex of a vertebral body. × 190.

FIG. 4. Radium rat 25634. Atypical new bone formation in the marrow cavity of a vertebral body. × 225.

3

4

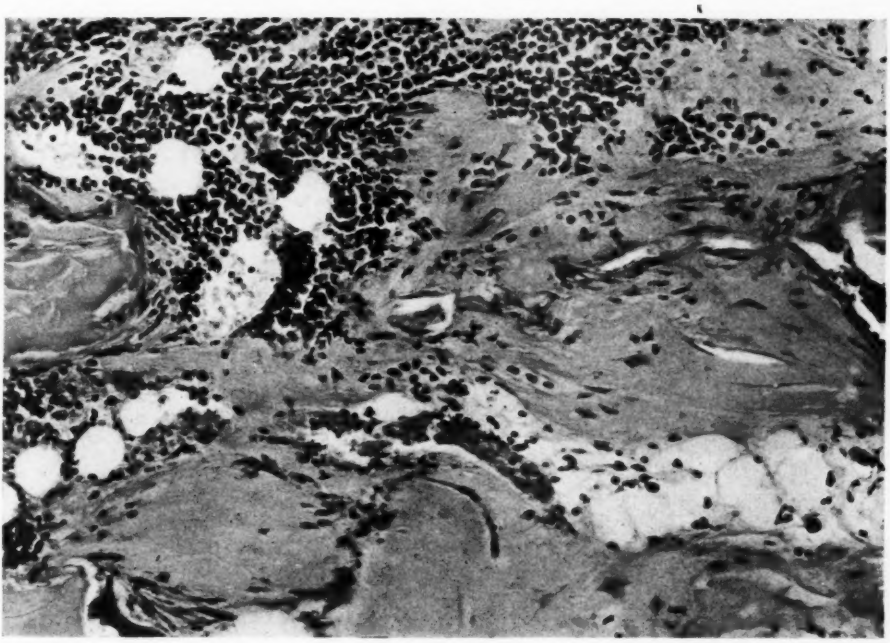

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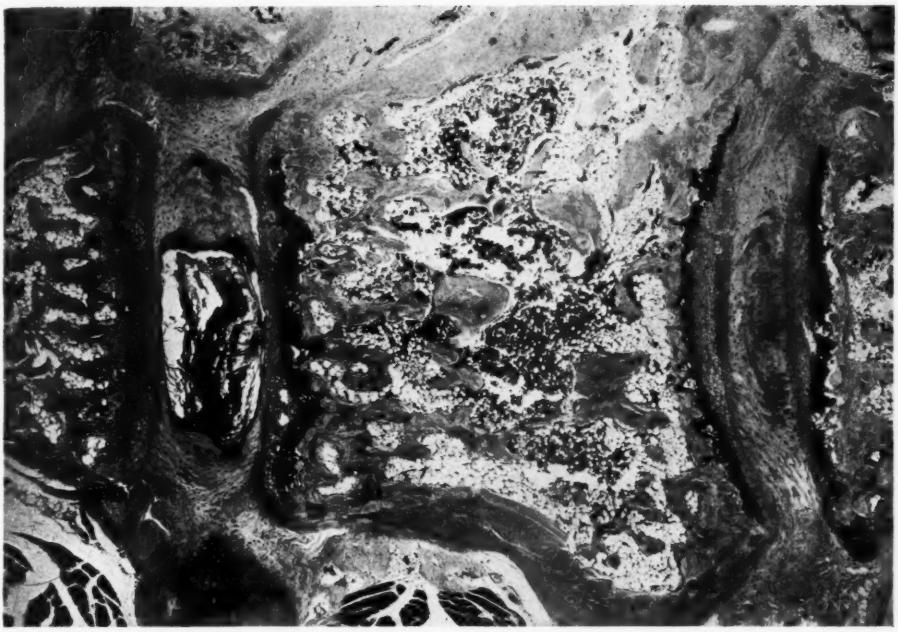
PLATE 3

FIG. 5. Radium rat 25630. Vertebral body showing peripheral atrophy of marrow and necrosis of cortical bone. × 21.

FIG. 6. Radium rat 25631. Alkaline phosphatase stain of tumor invading striated muscle. The distribution of alkaline phosphatase is indicated by the areas which are stained black. It is confined rather sharply to the tumor. × 70.

5



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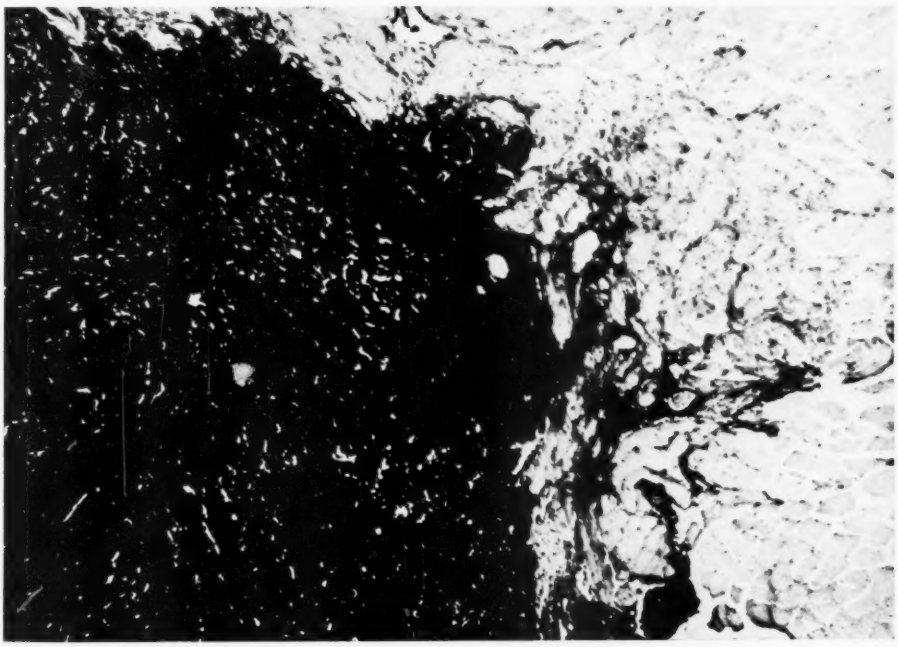

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ADAMANTOBLASTOMAS IN THE SLYE STOCK OF MICE *

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Previously, all studies dealing with the "adamantinomas" have depended upon material removed for biopsy, surgical specimens and postmortem findings, chiefly from human subjects. While a great deal of information was obtained in this way, certain limitations were apparent. Among these was the fact that the site of origin disappeared either through obliteration by the growth, natural inclusion into the tumor, or by other means such as damage during surgical intervention.

Animals, and more specifically rodents, constitute a better source of material because the entire head can be sectioned and studied, thus retaining all original relationships.

Wells,[1] in his Trimble lecture, stated that:

"There is no fundamental difference between the tumors which occur in man and those which occur in animals. . . . Cancer in mice appears in most of the forms seen in man, and in far greater variety than had previously been supposed. The importance of the demonstration of this abundant variety of tumors in mice lies in the fact that it establishes the identity of neoplastic disease of mice with that in man. Nearly all the tumors that are found behave in much the same way, occur at a corresponding period of life, in response to similar conditions, and present exactly the same histological structure as similar tumors in man."

Thus, because of the nature of the material as well as the number of animals available, I considered myself very fortunate in obtaining for this study a group of mice with epithelial tumors of the jaw. Many interesting aspects of the tumor problem presented themselves. However, I limited my efforts to a classification of central epithelial tumors of the jaw based on morphology and histology, and primarily to a search for the origin of these tumors.

SYNONYMS AND DEFINITION

"Adamantinomas" are referred to in the literature by various names. Among these are adamantinoma, adamantoma, ameloblastoma, enameloblastoma, cystadenoma adamantinum, epithelioma adamantinum, adamantinocarcinoma, adamantoblastoma, adenocarcinoma, cystosarcoma, multilocular cysts and, in some instances, follicular cysts or den-

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tigerous cysts. The more common of these names are: adamantinoma, ameloblastoma and adamantoblastoma.

Malassez,[2] in 1885, coined the term "adamantine epithelioma" to designate these tumors as epithelial in character and derived from enamel-forming tissue. In 1902, Borst [3] introduced the more generally accepted term "adamantinoma."

Adamantinoma and adamantoma are derived from the Greek ἀδάμας meaning "of the hardness of stone." In 1910, Galippe [4] maintained that the term "adamantinoma" signified a growth composed of formed enamel, *i.e.*, a tumor of adamantine structure. However, Kronfeld [5] pointed out that pure adamantinomas never contain enamel. The term "adamantinoma," therefore, meaning a tumor composed of and limited to adamantine structure, must be a misnomer since cells and structures other than those adamantine in form are often found.

Enameloblastoma signifies a tumor of enameloblasts, *i.e.*, already specialized enamel-forming cells. Although enameloblasts or cells simulating their features are sometimes observed, it is by no means the typical cell in all such tumors. This term should be discarded.

Ivy and Churchill [6] were the first to apply the name "ameloblastoma" to this group of tumors. Robinson,[7] in his review of 379 cases, has subscribed to its use saying that it is the most descriptive. He defined it as a tumor arising from the odontogenic apparatus, or from cells with a potentiality for forming tissues of the enamel organ.

Ameloblastoma, etymologically, means a tumor from or of enamel-forming cells. More precisely, it tends to convey the idea that the ameloblastic stage of development or the embryonal enamel-forming cell is the typical basic cell in all instances. This is not generally true.

The adamantocarcinoma or adamantinocarcinoma is defined as a malignant epithelial tumor of adamantine structure. Such cases have been reported in the literature. In this study, I have observed a group with definite carcinomatous characteristics but, since it comprised only a fraction of all the cases, I believe that the term adamantocarcinoma should be reserved as a subheading.

Epithelioma adamantinum is another term advocated to differentiate the malignant from the more benign "adamantinomas." This is a needless differentiation and, if used, should be a subdivision.

Multilocular cysts, follicular cysts and dentigerous cysts are purely descriptive terms from a morphologic standpoint. They do not convey information concerning origin or histologic structure. These terms simply tell us whether the tumor is unilocular or multilocular in structure and, if unilocular, whether or not a tooth is included in the growth.

Since all these terms are lacking in some particular, a more suitable

term is desirable. This name should include information concerning the principal cells of origin, with histologic and morphologic descriptive adjectives. Such a name, first suggested, to my knowledge, by Thoma,[8] is adamantoblastoma. Adamantoblastoma is the generic term which I accept and which I will use when referring to this group of tumors.

My definition of adamantoblastoma is a tumor which arises from the embryonal epithelial cells of the enamel organ. The unspecialized epithelial cells of the enamel organ are capable of forming any or all of a large variety of epithelial cell forms through their embryonic multipotentiality. The term adamantoblastoma is not limited in application to growths of any particular form of epithelial cell nor to any particular stage of cellular development, but rather includes all types which may be derived from the embryonic epithelial cells of the enamel organ. This definition is based on conclusions from the material which is to follow.

Literature with Reference to Origin

Broca,[9] in 1868, reported his observations upon the adamantoblastoma, although cystic tumors were already recorded in the literature at that time. Falkson,[10] in 1879, gave the first complete description of an adamantoblastoma. Since then, many opinions have been expressed as to the origin, malignancy and general nature of these growths.

From a clinical standpoint, interest in these tumors centers mainly about their possible malignancy. However, if the site of their origin could be determined, the problem of malignancy might be better explained. In the literature are found reports of many studies dealing with the search for the origin of adamantoblastomas and yielding a number of theories, all materially different. Thus, the adamantoblastoma was thought to be derived from:

(1) residual epithelial cell nests from (a) enamel organ, (b) Hertwig's sheath, or (c) dental lamina
(2) proliferative disturbance of the enamel organ or the tissue forming it
(3) mucous membrane epithelium of the jaw
(4) the epithelium of odontogenic cysts

The origin of adamantoblastomas occurring in remote structures such as the brain or tibia must also be considered in the light of independent growths.

Epithelial Cell Nests. Malassez,[2] in 1885, was the first to observe epithelial cells in the peridental membrance about the roots of teeth. These he called paradental epithelial débris, which, he continued, might become proliferative and give rise to epithelial growths in the

jaws, including "adamantinomas." This idea has been widely accepted; however, without positive evidence.

It is generally agreed that the remnants of the sheath of Hertwig and the dental lamina are the chief sources of these epithelial cell rests. Of greater importance is the origin of the Hertwig's sheath, which is still in dispute. Some state that it is derived from the outer multipotential epithelial cells of the enamel organ or from the inner specialized ameloblastic layer, or, from both. Orban [11] stated that it is formed from both. Diamond [12] maintained that it is derived from the outer cells of the enamel organ.

Epithelial cell nests are believed to be very common in the jaws. They may be found in the dental follicle before the tooth has erupted, in the adult periodontal membrane, and may occur in the marrow spaces adjoining dental crypts. Thoma [8] and Kotanyi [13] reported two cases of small adamantoblastomas in girls which developed between the teeth in lower jaws and presumably arose from epithelial rests in the periodontal membrane. Bauer [14] associated the adamantoblastoma with an excess of tooth germs as well as epithelial débris. Cysts, he stated, may develop by overgrowth of epithelial columns through degenerative processes within the epithelial strands. The uppermost epithelial débris is often connected with oral epithelium and so keeps the potential capacity to differentiate into varieties of cells.

Ewing,[15] in his book "Neoplastic Diseases," stated that the tumors of the "adamantinoma" group arise from paradental epithelial débris. Stout,[16] in his text "Human Cancer," wrote that, since the cysts and tumors that form in the jaws are inside the bone and in no demonstrable way connected with the alveolar epithelium in the early stages of their evolution, the presumption would be very strong that they arise from epithelial débris.

Hankey,[17] Masciottra,[18] Hayes [19] and Steensland [20] were of the opinion that adamantoblastomas arise from paradental epithelial rests. Murphy [21] cited statistics showing that the development of these tumors is not in relation with the development of the teeth and is thus opposed to the enamel organ theory and in harmony with that of aberrant epithelial rests. Krompecher,[22] in his report of 5 cases, thought that such tumors arose from paradental débris or embryonal rests but added that they could also arise from the oral mucosa. McFarland and Patterson,[23] after a critical review of 196 cases, reported in the medical and dental literature, concluded that adamantoblastomas arise in the jaws from the paradental epithelial débris.

In contradistinction, James and Counsell,[24] two prominent British investigators, stated that there is no reason to believe that the epi-

thelial rests play any part in the production of pathologic epithelium. Hatton [25] also said that there is little reason to consider that Malassez' epithelial débris has anything to do with the genesis of adamantoblastoma. The views of these latter authors will be cited again.

Enamel Organ. The enamel organ is often cited in the literature as the origin of adamantoblastomas. This designation is vague since this organ is composed of several strata, any or all of which may be considered the origin. It is composed of an inner layer of ameloblasts, the stratum intermedium and stellate reticulum, in that order, and an outer layer composed of embryonal epithelial cells. Few of the authors who consider the enamel organ the origin of adamantoblastomas state definitely which are the responsible layers. Broca,[9] Falkson [10] and Hatton [25] point to the enamel organ as the site of origin, each believing that these tumors arise from overgrowths of the dental germ, supernumerary tooth buds and dental lamina or tooth bud respectively.

Gentsch [26] maintained that true adamantinomas occur only in the jaws, since they arise from the enamel organ of developing teeth. Geschickter [27] observed adamantoblastomas in negro patients, many of whom had rickets. He concluded that this deficiency might have caused some disturbance in the enamel organ which went on to tumor formation. Kegel,[28] who studied histories of surgical cases from the Johns Hopkins pathological laboratory, came to the conclusion that adamantoblastomas arise from the cells of the enamel organ rather than from the paradental débris of Malassez. He regarded the inner epithelial layer as the origin rather than the outer and upper layers. The latter, he stated, give rise to the so-called epithelial débris.

Colyer and Sprawson,[29] on the other hand, believed that the adamantoblastoma does not originate from the enamel organ.

Mucous Membrane. The epithelial cells of mucous membranes have been considered a possible site of origin of adamantoblastomas. Colyer and Sprawson,[29] Büchtemann,[30] Eve,[31] von Bakay,[32] Kuru,[33] Kaufmann [34] and Gullifer [35] subscribed to this theory. Several of these investigators reported cases showing proliferations attached to the oral epithelium. Robinson [36] reported a case of peripheral adamantoblastoma composed of squamous, glandular and enamel-organ epithelium. This tumor developed on the lingual side of the ramus of the mandible but did not involve the underlying bone, hence the inference is that its origin was in the oral epithelium.

Odontogenic Cysts. That adamantoblastomas may arise from odontogenic cysts is another view held by some investigators. Churchill [37] described two cases in which dentigerous cysts of the jaws seemed to assume ameloblastomatous characters. Schroff [38] observed a case which

"consisted of a dilated cyst membrane that microscopically showed the structure of a follicular cyst. . . . A short distance from the cyst was a solid mass, which microscopically showed the structure of an adamantinoma." Cahn,[39] in a case report of a dentigerous cyst, observed mural swellings on the inner surface of the sac which, he contended, possessed the potentiality of developing into an adamantoblastoma. Hankey [17] described a multilocular cyst arising from a maxillary dental cyst. Kotanyi [40] believed that dental cystic membranes should be removed in their entirety to prevent them from giving rise to potential cancerous masses. During the healing process following removal of a follicular cyst by the partial Partsch method, Becker [41] observed epithelial sprouts, which upon histologic examination were of adamantoblastic character, in the pavement epithelium of the retained cyst sac. Wigdortschik [42] reported a case in which a cyst of the mandible was treated by the Partsch method and recurred a year later in the form of an adamantoblastoma. Morlet and Morlet [43] described a tumor in the lower jaw which presented all the intermediate stages between a cyst and adamantinoma. This case, they maintained, confirms the common origin of the two affections.

Remote Structures. Until 1892 it was quite generally accepted that the adamantinoma was to be found only in the jaws. Onanoff [44] at this time observed that certain epithelial pituitary gland tumors exhibited marked similarities to the adamantoblastoma of the jaw. Since then, many such tumors have been reported and it is now quite commonly agreed that they also fall into the category of adamantoblastomas. These occur also in the posterior wall of the pharynx in the region where the hypophyseal duct develops. They are often referred to as hypophyseal duct tumors or craniopharyngiomas.

According to Duffy,[45] the pituitary duct, which forms from the ectoderm of the oral cavity, gives rise to epidermal cell rests from which adamantinomas may arise. He distinguished these tumors from the cysts of Rathke's pouch which develop from the cleft between the anterior and posterior lobes and which are lined by ciliated epithelium. Duffy described two cases, one of an intracystic squamous epithelial papilloma arising from a rest of the hypophyseal duct in the anterior lobe and a second of a cystic suprasellar tumor with adamantinomatous characteristics developing from an infundibular squamous epithelial rest of the hypophyseal duct. McFarland and Patterson,[23] in their review of the literature, also concluded that adamantinomas in the hypophysis arise from squamous epithelial débris of the hypophyseal duct. Twenty-six cases of adamantinoma of the pituitary gland, two cases of adamantinoma in the tibia and one in the upper lip were re-

ported in this review. Drummond [46] described a case of infrasellar adamantinoma which developed from isolated paradental débris found along the floor of the sella turcica. It eventually grew by way of the sphenoidal septum into the vomer.

Wohl [47] described a very hard tumor in the upper lip not connected to the jaw which, on microscopic examination, presented the typical features of adamantinoma. (This case is included in the review by McFarland and Patterson.[23])

Fischer [48] and Baker and Hawksley [49] reported adamantinomas in the tibia. Both were believed to be primary. Wolfort and Sloane,[50] in their report of two cases of adamantinoma in the tibia, concluded that a fragment of epidermis must have been included in an embryonic malformation and that it was excited to proliferation.

Robinson,[7] in a review of 379 cases of ameloblastomas, found 7 cases of growths in the tibia. On the basis of his comprehensive study and in view of the current inadequate knowledge of the dento-cystic tumors, he concluded that etiologic considerations are, at best, founded only on theoretical grounds. In his opinion the adamantoblastomas arise from some part of the odontogenic apparatus or from cells with a potentiality for development into tooth-forming tissues.

Literature with Reference to Malignancy

One other feature of adamantoblastoma pertinent to this study is the question of malignancy. Some authors maintain that these growths are benign; others believe that they are benign but potentially malignant and still others are of the opinion that they are only locally malignant.

Bauer,[14] basing his opinion on microscopic findings, maintained that cystic adamantoblastomas are benign tumors. He considered the reported cases of metastases as doubtful. Robinson,[7] in his review of 379 cases, cited 17 in which evidences of malignancy were found. In 9, metastases were observed. In 119, recurrences were reported following removal. In many, the recurrence was traced to incomplete enucleation. He concluded that adamantoblastomas are benign and that the relatively small number with malignancy are atypical.

Lemaitre, Delater and Ardoin [51] considered adamantoblastomas to be locally malignant tumors because they possess the power of invasion into the surrounding tissues and are distinguished from the highly malignant neoplasms by the fact that they grow slowly and rarely show mitotic figures. In rare instances, these authors found that adamantoblastomas changed into frankly malignant neoplasms. They cited the case of a boy of 17 years in whom an adamantoblastoma recurred fol-

lowing its removal. The adamantine type of cell had changed into a form exactly resembling the squamous epidermal type with intercellular bridges. Masciottra [18] cited three instances of recurrences. Logsdon [52] stated that recurrence is common if the tumor is incompletely removed. Moore [53] described a case of a man, 38 years of age, who had eight recurrences in the mandible in 7 years. Finally, the jaw was resected. At the last appearance a recurrence was observed in the stump. Frantz and Stix [54] reported several recurrences of an adamantoblastoma over a period of 51 years.

Ghosh [55] described the case of a boy, 18 years old, in whom a partly solid and partly cystic adamantoblastoma invaded the antrum. Broders and MacCarty [56] believed that, histologically, the adamantoblastoma closely simulates the squamous cell carcinoma. Oinoue [57] presented a case of adamantoblastoma primary in the mandible which metastasized to the lung, causing the death of the patient. Vorzimer and Perla [58] also had a case which metastasized to the lung but here they considered the possibility of aspiration during operation. Ewing [15] reported two cases with metastases. In one, small metastases were found in the lung and cervical lymph nodes, and in the other a fourth recurrence was in the cervical nodes and loose tissues of the neck.

Dorrance [59] emphasized the potential malignancy of adamantoblastoma. Spring [60] stated that recurrences are frequent in adamantoblastoma and that these tumors are known to develop the histologic characteristics of carcinoma. Geschickter [27] looked upon adamantoblastomas as neoplastic and potentially malignant homologues of follicular cysts.

Hatton [25] considered the solid adamantoblastoma as malignant. He stated that the multilocular cysts are equally malignant since they both tend to infiltrate and recur after excision. Sprawson and Keizer [61] contended that the adamantoblastoma should be designated as basal cell carcinoma on the basis of histologic study and malignant properties. Dew and Miller [62] maintained that these tumors possess the aggressive properties of epitheliomas of local malignancy.

Simmons,[63] in his report of 12 cases of adamantoblastoma, maintained that, although it is usually true that these tumors are benign, they are potentially malignant. He cited 2 cases with glandular metastases. These metastases occurred late, were regional at first and, in 1 case in which they were later general, dissemination was not demonstrated until 14 years after the onset of the disease. In the second case metastases in the glands of the neck appeared 12 years after the original tumor. Sections of the primary tumor and the metastatic glands confirmed the primary-secondary relationship. Conservative

operations were performed in 10 cases and in each instance there was a recurrence. Two patients died from local extension of the disease and sepsis and 1 of metastasis.

From this summary of the literature it will be seen that adamantoblastomas are classified as benign tumors by many writers. The majority, however, believe that they should be grouped with the neoplasms of local malignancy, since they invade and destroy the surrounding bone and soft tissues and, when handled conservatively by surgical means alone, they often recur. Moreover, there is general agreement that these tumors possess the property of potential malignancy. The classification of frank malignancy is reserved for those rare adamantoblastomas which have demonstrated their potential malignancy by metastasizing to neighboring lymph nodes, soft tissues of the neck, lungs, or other structures.

Embryology, Histology and Anatomy of the Rodent Incisor

Since this study is based upon incisor teeth of mice and is concerned particularly with the formative enamel organ, review of the normal embryology, histology and anatomy is essential.

The incisor teeth of rodents are continuously growing and erupting structures, but in their functional capacity they wear away so that the teeth in normal adults remain of constant size. The rate of wear, then, equals the rate of eruption and formation. The failure of an incisor to occlude normally with its antagonist results in an elongation unchecked by wearing. The normal rate of eruption is about 2 mm. for the upper and 2.8 mm. for the lower teeth per week. The teeth renew themselves completely every 35 to 45 days. These teeth grow by apposition of dentin at the pulpal surface and by elongation of the basal part where is found a widely open apical foramen. The dentin comprises the bulk of the tooth. The enamel is formed only on the labial or convex side of the tooth and overlaps slightly the lateral surfaces. The remaining part of the tooth is covered with cementum.

Enamel is not formed over the tip of the tooth at any time. Before eruption this area is filled with so-called "osteodentin" produced by the underlying pulp cells. As the tip wears away with use, the pulp forms more of this substance, which has been called secondary dentin, so that the pulp is never exposed.

The dentinal papilla, composed of dentinoblasts, is situated at the posterior end of the developing and erupting incisor. These dentinoblasts are the first cells to differentiate, laying down dentin shortly before the enamel organ becomes functional to produce enamel. External and inferior to the dentinal papilla lies the basal end of the

formative enamel organ. Here, a mass of closely packed embryonic epithelial cells give rise to its cellular structures. These cells are functional throughout the life of the animal in accordance with the persistent growth of these teeth, *i.e.*, the organ is not transitory as in man.

The enamel organ has fundamentally the same four layers as seen in man. The inner layer consists of distinct tall columnar ameloblasts, a stratum intermedium of two or three layers of flattened cells and a stellate reticulum of loosely arranged cells. The outer layer consists of a single row of cuboidal cells which sends out papilla-like buds into the connective tissue. Numerous blood vessels of the latter occupy the depressions between the epithelial elevations. Toward the middle portion of the labial surface, the enamel organ reaches its highest functional development, the ameloblasts are tallest and the stratum intermedium consists of one or two rows of cuboidal cells. The stellate reticulum loses its characteristic appearance and seems to be replaced by the outer enamel epithelium, which is now in contact with the stratum intermedium. The papillae thus formed are more prominent and considerably higher than at the basal end, and are surrounded by an abundant capillary blood supply.

As the tooth erupts, the ameloblasts move forward at an equal rate and recede labially with the apposition of organic enamel matrix.[12] Enamel formation starts at the basal end and increases in thickness, becoming more fully calcified as it extends anteriorly. In the rat the normal daily rate of enamel formation is 16 μ.

A very thin layer of cementum covers the lingual, mesial and distal surfaces, gradually increasing to a thickness of 3 to 4 μ toward the incisal end. The peridental membrane is comparatively wide and has a rich blood supply.

As the enamel organ proceeds forward with the erupting tooth, regressive and degenerative changes occur in it. The ameloblasts gradually become shorter and cuboidal, losing their power of amelogenesis. The stratum intermedium and the external layer of epithelial cells also show regressive changes fusing with the degenerated ameloblasts. The resultant structure takes on the appearance of a flattened stratified squamous epithelium, joining with the squamous cells of the gingivae at the oral junction.

Functionally, the rodent and human enamel organs serve the same purpose, formation of enamel. The only important difference revolves about the property of the rodent incisor of continuously growing and erupting, as differentiated from the human tooth which has a limited developmental stage. Whereas the basal enamel organ and formative dentin cells in the rodent incisor proliferate throughout life, in man, at

the completion of enamel formation and before eruption, the enamel organ degenerates. This functional difference in the two enamel organs is not as important as it may seem, since the onset of tumor formation in man must occur before degeneration of the enamel organ takes place in order to comply with the views already stated citing the enamel organ as the site of origin of adamantoblastomas. For this reason, as well as others already stated, the microscopic description of the origin of these growths in mice may, with equal reasonableness, be applied to man.

Material

The material for this study was a group of 79 mice (Table 1) obtained from the Maude Slye colony at the University of Chicago. Only animals were chosen in which tumors of the jaw were present. All growths were found in mice wherein heredity was the factor under study. I will not attempt in this paper to delve into the genetic problem involved. This has been admirably stated by Wells, Slye and Holmes.[1, 64-69]

Efforts had been directed toward providing balanced diets and good hygienic conditions, and the mice were allowed to live their full life span.

Methods

After natural death, the heads of these animals were removed and placed in a 4 per cent formaldehyde solution and allowed to remain there for periods varying from 3 months to 2 years before being acquired by me. My first procedure was to record gross descriptions; then, 25 heads were sawed anteroposteriorly in the midsagittal plane for the purpose of taking x-rays. By this method it was found that both halves could be radiographed more accurately. The remaining heads were radiographed intact. All radiographs were taken at 20 inch target-film distance, using 50 kv. and 10 ma. of current. Exposure time was 1.5 to 2.0 seconds. The heads were then decalcified in a 5 per cent nitric acid solution in which the diluent was 4 per cent formaldehyde. Daily changes of the solution were made until the ammonium oxalate test showed that the decalcification was complete, the average length of time being 72 hours.

The specimens were then washed in running water for 24 hours and dehydrated in the alcohols in the usual manner. They were then placed in 4 to 5 per cent celloidin and tightly sealed, where they were allowed to remain for an average of 4 months. The next change was to 10 per cent celloidin for 1 month and then to 14 per cent celloidin for another month. Finally, they were laid in small Stender staining dishes containing 14 per cent celloidin, allowing slow evaporation and concentration

TABLE I

Seventy-nine Mice from Maude Slye Colony of University of Chicago
(Animals Arranged According to Findings)

A. ADAMANTOBLASTOMAS	CONCOMITANT FINDINGS (Findings in opposite side of mandible in same animal)
1. MONOCYSTIC	
(a) Monolobulated	
2	Multicystic
5	Monocystic
6	Normal
7	Normal
8	Monocystic
12	Multicystic solid
13	Multicystic epidermoid
16	Multicystic epidermoid
21	Sarco (giant cell)-adamantoblastoma
30	Monocystic
31	Monocystic
32	Sarco (osteogenic)-adamantoblastoma
33	Monocystic
34	Monocystic
36	Monocystic multilobulated
37	Monocystic
41	Monocystic
42	Monocystic
43	Multicystic solid
44	Sarco-adamantoblastoma
48	Monocystic
49	Normal
52	Normal
55	Multicystic solid
57	Multicystic epidermoid
58	Multicystic epidermoid
59	Multicystic epidermoid
60	Multicystic epidermoid
61	Monocystic
62	Multicystic epidermoid
63	Monocystic
65	Multicystic epidermoid
67	Multicystic solid
68	Monocystic
69	Multicystic
70	Multicystic epidermoid
71	Solid epidermoid
72	Multicystic epidermoid
(b) Multilobulated	
10	Normal
15	Normal
19	Multicystic epidermoid
36	Monocystic
2. MULTICYSTIC	
(a) Without solid masses	
2	Monocystic
35	Multicystic
39	Multicystic
50	Multicystic solid
54	Sarco (spindle cell)-adamantoblastoma
64	Multicystic
73	Monocystic
74	Normal
75	Normal
76	Normal

TABLE I—*Continued*

(b) Multicystic solid	
12	Monocystic
20	Multicystic solid
43	Monocystic
50	Multicystic
55	Monocystic
66	Multicystic solid
67	Monocystic
(c) Multicystic epidermoid	
9	Normal
13	Monocystic
16	Monocystic
19	Monocystic multilobulated
38	Multicystic epidermoid
56	Normal
57	Monocystic
58	Monocystic
59	Monocystic
60	Monocystic
62	Monocystic
65	Monocystic
70	Monocystic
72	Monocystic
77	Normal
3. SOLID EPIDERMOID	
40	Normal
51	Normal
71	Monocystic
B. SARCO-ADAMANTOBLASTOMAS	
1. SARCO (OSTEOGENIC)-ADAMANTOBLASTOMA	
4	Bilateral
32	Monocystic
2. SARCO (SPINDLE CELL)-ADAMANTOBLASTOMA	
17	Bilateral
54	Multicystic
3. SARCO (GIANT CELL)-ADAMANTOBLASTOMA	
21	Monocystic
4. SARCO (FIBRO)-ADAMANTOBLASTOMA	
44	Monocystic

MISCELLANEOUS FINDINGS

Sarcoma	
1	Giant cell
3	Fibro-
14	Spindle cell (in cheek)
18	Osteogenic—odontoma
22	Osteogenic (in both mandible and maxilla)
45	Osteogenic
65	Round cell
78	Osteogenic
69	Osteofibroma
Odontoma	
11	Odontoma
18	Sarco (osteogenic)-odontoma
Others	
46	Multiple abscesses of tongue and jaws
47	Extra-mandibular growth
53	Ranula
79	Multiple abscesses

to take place. More celloidin was added as needed to cover the exposed tissue surfaces. The specimens were then removed to chloroform desiccators for final hardening, and stored in 70 per cent alcohol until ready for sectioning.

Serial cross sections or longitudinal sections, 10 μ thick, were cut. Every tenth section was mounted and stained with a dilute solution of Harris' hematoxylin. Sections were allowed to remain in this solution overnight, after which they were counterstained with eosin. The intermediate sections were stored in 70 per cent alcohol and used only when needed.

Special stains were used in all cases where any doubt existed as to the correct diagnosis or where more accurate differentiation of tissues was desired. The most commonly used special stain was the Heidenhain's azan stain.

Observations

Gross Pathology

The heads of the animals were grossly distorted by the tumors, some of which were firm, others soft, while the usual finding of crepitation was a prominent feature in the larger growths. These expanded laterally to such an extent as to almost double the normal horizontal width of the face. Others protruded from the under surface of the mandible and greatly displaced the lower jaw. Still others involved both halves of the mandible while a few were bounded by the orbit superiorly, the external auditory meatus distally, the midline mesially and the lower border of the mandible inferiorly.

In all cases, the incisor teeth only were involved in the growths. In most of these, the incisors were forced out of their natural positions so that the resultant malocclusion prevented normal wear. Thus the teeth attained extreme lengths and varied shapes, protruding from or into the mouth abnormally.

In many instances these displaced teeth penetrated the cheeks, opposing jaws, tongue and nasal cavity, causing soft tissue abscesses and osteomyelitis. In several animals in which the tumor mass extended into the mouth, difficulty in eating resulted in inanition. In others, mastication caused surface abrasions which developed into ulcerations with secondary infection. In some cases, the infection eventually caused the death of the animal.

Roentgenographic Examination

Radiographs of the head of each animal were taken from two different angles before decalcification. Intra-oral dental films were used. The tumors presented a variety of appearances. Monocystic involve-

ment was most frequently observed (Fig. 2). Multicystic tumors, with thin radiopaque lines dividing the lobules, were next in order of frequency. Growths giving the homogeneous radiolucent appearance of soft tissue were also observed. The latter proved to be solid epithelial tumors.

The molar teeth were displaced by the expanded tumors. In the radiographs, crowns of the distorted incisor teeth were greatly elongated, and took on the form of concentric circles or sectional arcs (Fig. 1). The roots of a few were found to be shortened, but only in teeth involved in the growths. The normal structural outlines of the mandible on the side of the growth could not be seen because, as the growth expanded, the bone also expanded and thinned out or was lost entirely (Fig. 2). Portions of the growth extended into the ramus of the mandible, often obliterating it completely. Posteriorly the tumors were observed to extend distally to the ear (Fig. 1).

Microscopic Pathology

The adamantoblastomas of my series were found to arise in the mandible only. This does not exclude the maxilla as a possible site. These tumors were intra-osseous or central and extended over wide areas, causing great destruction and displacement of normal structures. The normal bony outlines of the affected mandibles were completely lost, especially in the more posterior regions where the maximum size of the tumor was most often found (Figs. 3, 4 and 6).

Displacement of the lower incisor teeth of the involved jaws was a common finding (Figs. 4 and 6). In most instances the entire tooth was bodily displaced from its normal position toward the midline and superiorly by the expansive force of the tumors. The growths in these animals were inferior to the teeth, below the enamel space (Figs. 7 and 8). Distortion of tooth contour by tumor pressure was observed quite frequently but the intrinsic structural development of the enamel, dentin and cementum was not disturbed (Fig. 4). The molar teeth of the involved jaws showed no abnormalities but that of change in position caused by tumor pressure. The base of the tongue was invaded by neoplastic tissue in several cases, and the tongue itself was grossly displaced (Fig. 42).

In one animal, epithelial strands from the main tumor mass invaded the root of an incisor tooth, proliferating widely in the pulp chamber and canal. This epithelial mass was seen to break down and form a fairly large cyst in the body of the tooth which, by its outward pressure, caused complete resorption of the dentin, enamel and cementum in the formative portions of the tooth.

Many animals showed invasion of the ramus with replacement of the mandible by neoplastic tissue (Figs. 4, 6 and 41). Resorption and regeneration of mandibular bone were observed in all involved jaws. Maintenance of some semblance of a bony periphery around the tumor masses was observed, but even this thin shell was often absent so that only a narrow layer of fibrous connective tissue and elongated muscle fibers formed the external limits of the cyst (Figs. 4 and 6). In some mandibles little or no normal bone marrow was found due to its resorption, while in others fibrous connective tissue or invading epithelial masses filled the marrow spaces.

The striated muscles of the jaws were found to be lengthened and degenerated. In a few instances, only small remnants remained to indicate their presence in these regions (Figs. 4 and 6).

In eight animals, because of the extent of the mandibular growths, traumatic injuries occurred resulting in infections and suppurations. Oral ulcerations were observed spreading into the central portion of the tumors. Secondary infections were also seen in isolated extra-oral areas of these adamantoblastomas.

For purposes of further description, I have divided the tumors according to their (1) cystic or (2) solid character, some being composed of cystic structures only, while others were solid, and still others, both cystic and solid in different areas.

Cystic Structure

The linings of most cysts were composed of stratified squamous cells varying from two to five cells in thickness (Figs. 5 and 6). The cells were of the usual squamous variety with intercellular bridges. In many instances, however, the cellular linings were greatly distended with consequent flattening, forming thin, dark lines (Fig. 9). In such cases they were stretched to double and triple their normal lengths and flattened to one-third to one-fourth of their normal thickness. The remaining portion of the cyst wall was composed of a layer of fibrous connective tissue consisting in some cases of thick collagen fibers, in others of thin fibers which were arranged parallel to the periphery. Where bone was seen external to the cyst wall, it was compact, showing evidences of resorption on its internal surface and new bone formation on its periphery.

The contents of the cysts varied, but this did not seem to differentiate one type of adamantoblastoma from another. The composition of the cystic contents included: clear cystic fluid, cholesterol, foam cells, desquamated epithelial cells, inflammatory cells (round, polymorphonuclear, and plasma cells), hemorrhagic material, hemosiderin granules,

and keratohyaline structures. The cystic cavity was often entirely filled with clear fluid having a finely granular texture. Frequently clear fluid formed the bulk of the contents with various amounts of the other constituents and with no particular specificity (Fig. 6). Cholesterol slits (Fig. 5) and foam cells were often observed but always constituted a minor proportion of the contents. Desquamated epithelial cells (Fig. 9), usually in small numbers, were found close to the cyst membrane. In some instances the cystic space was entirely or partially filled with inflammatory accumulations (Figs. 5 and 31). Hemorrhage was occasionally observed, with red blood cells clearly distinguishable, little degeneration being present. In others, hemorrhagic débris in the form of clumps and densely granular structures was present as were phagocytic cells with ingested hemosiderin granules (Figs. 4 and 41). Keratohyaline structures were seen only in certain of the tumors. In some of these, keratin completely filled the cyst while in others it was found in varying degrees of dissolution (Figs. 7 and 10).

The keratohyaline structures in my group occurred in the multicystic epidermoid adamantoblastomas.

Solid Epithelial Structure

The solid epithelial portions of these growths varied in quantity and in cell type. Dense, broad sheets of epithelium with strands, cords and columns of cells, frequently anastomosing and branching, permeated the stroma. Central breakdown of such epithelial extensions was commonly seen with the degenerative masses tending to form other cysts (Figs. 7, 10, 11, 12, 13 and 14).

In a given tumor, the majority of cells were of one distinct type, but it was common to find all degrees of differentiation. The variations ranged from undifferentiated epithelium to differentiated stratified squamous epithelium or specialized adult ameloblasts. Epithelial cells and structures observed included: stratified squamous cells, epithelial pearls, adenomatous structures, stellate cells, adamantine-like cells, basal cells, embryonal syncytium, sarcoma-like epithelial cells. The stratified squamous cell was the most frequently observed type (Fig. 11). Broad sheets of such cells often formed large portions of the solid areas of adamantoblastomas. Intercellular bridges were clearly distinguishable in most cases (Fig. 11). Anaplasia was minimal (Figs. 12 and 15). The nuclei were large, hyperchromatism was infrequent (Figs. 13 and 14) and mitotic figures were rare (Fig. 38). The cytoplasm was granular and abundant. The branching and anastomosing of these epithelial cells gave the general microscopic appearance of a squamous cell carcinoma (Figs. 32, 35, 36 and 37).

Many variations among the epithelial cells were noted in most of the tumors. In some instances these variations comprised considerable portions of the tumor mass. Epithelial pearls in the form of bulbous masses, with concentric arrangement, were observed. These structures presented all stages of keratinization in passing from the outer layers of cells toward the center. The peripheral layers of such pearls were of the prickle cell type; keratohyaline granules appeared in cells of the inner layers, with gradual transformation into structureless masses of keratin. The epithelial pearls presented varying sizes and shapes, some being huge with large amounts of keratin while others were quite small. Also seen were large pearls in various degrees of dissolution (Figs. 7 and 10).

Gland-like arrangements of cuboidal and columnar cells without positive secretory activity were seen as isolated masses completely surrounded by stratified squamous cells (Figs. 12 and 13). These adenoma-like structures were not completely differentiated as such but assumed irregular alveolar arrangements.

Cells of the enamel organ type made up portions of the epithelial structures of the adamantoblastomas. The stellate stage of epithelial development was often found (Fig. 12). These cells appeared in isolated regions in groups of varying size. Resemblance to the stellate reticulum was further emphasized by the cells forming a latticework arrangement because of the joining of cytoplasmic strands. The intervening spaces were clear and filled with a mucoid material.

The highest degree of differentiation of epithelial cells was attained by those cells which were adamantine or enameloblastic in form. These cells were of the tall columnar type with hyperchromatic nuclei, irregularly situated in the densely stained cytoplasm. Such areas were small and infrequent. Although the individual cells resembled enameloblasts, the groups did not follow the enamel organ pattern. In those instances where areas of "stellate reticulum" and "enameloblasts" were seen, it was noted that most of them were separated and in no way functionally related to each other. Although the enameloblastic stage of development was reached by isolated groups of cells, the enamel-forming function was definitely lacking. *No formed enamel was observed.*

Basal cell epithelium (Fig. 14) in strands and columns frequently constituted considerable portions of the solid epithelium. The cells contained little cytoplasm and were smaller than the squamous variety. The nuclei stained densely with hematoxylin. Masses of degenerated basal cells with small cysts were seen in isolated areas. No keratinization was observed.

Areas of embryonal epithelial syncytium were seen in a small number of tumors (Fig. 15). The cells were undifferentiated, cuboidal in form

and widely separated by a pale, blue-staining material, mucoid in appearance. Occasionally the cytoplasmic strands joined and presented a uniform appearance.

In several instances, epithelial cells in broad sheets arranged themselves in patterns simulating spindle cell sarcoma (Fig. 16). The cells were elongated, with small amounts of granular cytoplasm and large, hyperchromatic nuclei. Intercellular bridges were indistinct. Mitotic figures were frequent. These masses of cells widely infiltrated the neighboring regions and surrounded portions of the connective tissue stroma and isolated bone spicules in the process of resorption.

Stroma

The amount of matrix varied considerably in different tumors. Those which were largely cystic contained small amounts of stroma, while the solid and partially solid adamantoblastomas usually contained large quantities. The thin strips of dense fibrous tissue between the cyst wall and the periphery of the purely cystic growths were compressed and avascular, and were found both with and without bony septa.

Three types of stromas were observed, heterogenously distributed. In the first, hyalinized collagenous bundles predominated; the second was composed mainly of cellular fibroblasts, and the third was mostly sarcomatous in nature. The bundles of hyalinized collagen were most often seen. These appeared as dense, eosin-stained masses with narrow fibrocytes flattened between them (Figs. 17 and 18). Isolated epithelial infiltrations in the form of nests and narrow strands were often found surrounded by this collagen. In some tumors, where the epithelial tissue was abundant, small islands of collagen were surrounded by strands of epithelium in lattice-like arrangement (Fig. 13).

The second type of stroma with large, prominent fibroblasts (Fig. 19) was well vascularized. Networks of newly formed capillaries coursed through this stroma resembling granulation tissue (Fig. 21). Fibrous connective tissue was minimal, with only short, thin strips separating the fibroblasts. This is an embryonal type of stroma.

The third main type of matrix—sarcomatous—was found in only a few instances (Fig. 20). However, whenever it did occur the problem arose as to whether these were true sarcomas with some epithelial proliferation or adamantoblastomas with a sarcomatous matrix caused by a pronounced desmoplastic property. This problem was emphasized because, in several instances, definite mixed tumors (sarco-adamantoblastomas) were found. The predominating features of this type of matrix were: (1) areas of elongated spindle cells evenly arranged and uniform in size, forming the bulk of the tumor; (2) scanty intercellular substance with the cells close together; (3) cross section of bundles

appearing as round cells; (4) matrices more dense and more widely invasive locally, but limited peripherally by epithelial borders. While these four features might with equal accuracy be utilized in describing a sarco-adamantoblastoma (Fig. 22), my principal means of differentiating these as adamantoblastomas with sarcomatous matrices was in the degree of anaplasia and in peripheral limitation. Other findings in the stroma were: bony trabeculae and septa, remnants of resorbing bone, osteoclasts, new bone formations, infective and suppurative processes and hemorrhagic material.

Lobules of a single cyst and the individual cysts themselves were often separated from each other by bony trabeculae (Figs. 6 and 9). The thin septal layers of bone appeared compressed and were surrounded by small amounts of fibrous connective tissue. These bony structures were composed of fused, parallel layers, staining with varying degrees of intensity with hematoxylin. Osteocytes were absent or only faintly visible. Scattered in the stroma of some tumors were spicules of bone, the remnants of resorbed bone. These densely stained structures appeared in different shapes and sizes surrounded by fibrous connective tissue. Osteoclasts, in Howship's lacunae, were often observed. These large multinucleated cells were seen in some areas where active resorption of bone was apparent (Fig. 13).

Bone regeneration was a prominent feature in most tumors. The new formations of cortical bone often comprised considerable areas of the stroma. Usually they were located external to the central region of the adamantoblastoma as if to form the peripheral boundary of the tumor (Figs. 4 and 17). The new bone marrow spaces were filled with a vascular fibrous connective tissue in most cases but in a number of instances epithelial invasions from the main tumor mass penetrated deeply into these outlying structures (Fig. 35).

Infective and suppurative processes were found frequently, invariably arising from oral ulcerations or external jaw injuries (Fig. 42). These secondary infections were made evident by masses of round cells and polymorphonuclear leukocytes which sometimes occupied considerable portions of the entire tumor. The true picture of the tumor was often disturbed by the suppurative process which caused widespread liquefaction and necrosis. Recent and older hemorrhages were seen, with hemorrhagic débris and hemosiderin in the cytoplasm of large phagocytes (Fig. 21).

Vascularity

There was marked variation in the vascularity of these tumors. In the densely fibrous and hyalinized stroma, vascularity was minimal (Fig. 18). Although narrow, flattened blood vessels were observed be-

tween the bundles of collagen, they were few in number. The young fibroblastic matrix contained rich networks of capillaries and lymph channels. The structural endothelial cells were large and young in appearance. Numerous capillary anastomoses were observed coursing through the collagen-free stroma. Several tumors were observed which were rich in vascular channels. These were considerably oversized and close together, with little stromal material between them. The similarity to hemangiomas was striking but since these areas were purely local, occupying comparatively small portions of the main tumor mass, they were considered regions of atypical vascularization (Fig. 21).

Origin

The chief theories as to the origin of adamantoblastomas are three in number: (1) epithelial rests of Malassez; (2) mucous membrane of the mouth; (3) enamel organ. Of these, that of epithelial rests is the most favored. However, of 97 adamantoblastomas, only 5 cases, or about 5 per cent, were observed with epithelial rests in the peridental membrane, while of 30 normal mice, 2, or about 6 per cent, had epithelial rests in the peridental membrane. (Fifteen others were either mutilated for biopsy or had other tumors.) The epithelial rests were all inactive and purely local in character. In no instance was evidence of proliferation from epithelial rests seen. Except for a few tumors which completely encircled the incisor teeth, all were found on the *convex* side of the tooth far removed from the epithelial débris in the peridental membrane which covers the *concave* surface of the tooth (Figs. 23 and 24).

The next most popular theory attributes the origin to the enamel organ (Fig. 25). However, it has already been pointed out earlier in this paper that where the enamel organ is cited as the point of origin, the usual reference to it is vague in that it is considered in its entirety rather than as to any one of its four component layers. *In this study it was found that the enamel organ is unquestionably the site of origin of most of these adamantoblastomas and probably in all of them* (Figs. 23, 26, 27, 28, 29, 30, 31 and 32). Thus, of 97 adamantoblastomas, 87 showed unquestionable enamel organ origin with no other source seen. In 8, downgrowths of epithelium along the sides of oral ulcerations appeared to supply part of the tumor masses with what might be considered another site of origin. The remaining 2 were mutilated in obtaining material for biopsy.

In considering the particular stratum of the enamel organ which might be responsible for the origin of these tumors, I wish to point out especially the outer layer of epithelial cells (Fig. 25). These cells are

embryonal and unspecialized, retaining multipotential properties characteristic of embryonic epithelium. Proliferations of these outer cells of the enamel organ did occur, and were seen to form dense massive accumulations of epithelial cells external to the enamel space (Figs. 7, 8, 26, 27 and 29). The cells were small and their nuclei hyperchromatic, resembling at times a basal cell carcinoma. These proliferative masses retained their attachment to this outer layer. In some cases, the attachments were as thin as 0.1 mm. in width, and in others as wide as 6 mm. (Figs. 28 and 29). Several tumors presented interrupted attachments (Figs. 29 and 30). In most tumors, the attachments were found somewhere along the anterior half of the unerupted portion of the incisor tooth, with the main tumor mass posteriorly and below (Figs. 7 and 8). In the others the attachments were posteriorly, or anteriorly as far as the enamel organ-gingival junction. The width of the attachments from mesial to distal varied from one quarter to all of the convex-shaped enamel organ (Figs. 28 and 29). *Thus, the unspecialized epithelial cells forming the external layer of the enamel organ were determined to be the primary origin of all adamantoblastomas in this series* (Fig. 32).

The rôle of the gingivae and oral mucous membrane as points of origin was also considered. In no instance was there seen proliferation and downgrowth of the gingivae, except in the eight cases of oral ulcerations already cited. Even in those cases in which the site of attachment of the tumor was far enough anterior to be in contact with the oral gingivae, the identity of the attachment to the enamel organ was clearly retained. However, where oral ulcerations occurred, the growths appeared contiguous to the oral epithelium. Thus, it may be considered as a possible point of origin, but since the attachment to the enamel organ was also observed in all these tumors, the oral mucous membrane should be considered as a secondary site of origin.

Malignancy

In considering malignancy, I was guided by the criteria laid down by Ewing.[15] These are:

1. The presence of metastatic lesions in the neighboring lymph nodes
2. Invasions and infiltrations into the surrounding regions
3. Local destructive properties
4. Local interference with function

Metastases. Metastases from the primary growth in the mandible to the submaxillary lymph nodes were observed in but two animals (heads only available) (Figs. 33 and 34). The parent tumor of one was a

multicystic, solid adamantoblastoma with carcinomatous features and the other was a multicystic epidermoid type of adamantoblastoma. The involved lymph nodes were markedly enlarged; mostly filled by the metastases, and the remaining lymphoid tissue appeared compressed. In general histologic features the metastases were reproductions of the parent tumors. The cells were small, with round, hyperchromatic and densely stained nuclei and little cytoplasm. Intercellular bridges were few, difficult to identify, and were observed in small isolated areas only.

Invasion. Infiltration of surrounding tissues was frequently observed in the types of adamantoblastoma which contained solid epithelial portions. Long strands from the epithelial masses infiltrated the bone marrow spaces, obliterating the normal structure (Figs. 35 and 36). These were seen to anastomose and branch, frequently surrounding spicules of old bone or portions of the fibrous connective tissue. Thin columns of epithelial cells, cut at right angles, looked like large giant cells in bone marrow spaces but special staining confirmed their true epithelial structure. Epithelial cords, two to three cells thick, were seen extending into the neighboring structures distant from the main tumor mass and surrounded on all sides by dense connective tissue. Infiltrations into tissue spaces and occasionally into lymphatics by contiguous growth were observed. Five animals showed tumor infiltration into the connective tissue and muscles of the cheek (Fig. 37); in four, the base of the tongue was invaded and in one the unaffected side of the mandible became involved, all by direct extension.

Local Destructive Properties. Expansion and distortion with resorption of the jaws were more characteristic of the cystic adamantoblastomas. Thin layers of cortical bone, in which evidence of pressure atrophy could be seen, surrounded the cysts in some cases. In others, the cystic expansions caused complete resorption of all peripheral bone. The little remaining soft tissues of the stroma were so compressed by the expanding forces of the cysts as to be difficult to recognize. Membrane linings were flattened and distended. The cheek and the muscles of the jaws were stretched to several times their normal size (Figs. 4, 6, 17, 40 and 41).

Local Interference with Function. The expansion of these tumors almost invariably caused displacement of the incisor and molar teeth, producing severe malocclusion. The tongue was forced into grossly abnormal positions. The tumor masses invaded the temporomandibular articulation and caused extensive destruction in these areas. These destructive invasive forces, together with the disabling effects on the muscles, greatly contributed to limiting function or even to complete loss of function of the jaws (Fig. 6).

Thus we see that if we insist that all four of the criteria laid down by

Ewing be present to pronounce a tumor "malignant," only 2 of the 97 pure adamantoblastomas may be thus classified. About one-third lacked only the metastatic feature to be truly "malignant," while about two-thirds lacked two of the four necessary requirements to be classified "malignant." These findings cast doubt on the oft repeated statement that the adamantoblastomas are benign.

Mitoses. Mitotic figures were not observed in the purely cystic types. The epithelial cells were well differentiated and bore no anaplastic qualities. In the more solid adamantoblastomas mitoses were few, and were observed only after careful search (Fig. 38). In the pure solid adamantoblastomas, while anaplasia was a prominent characteristic, only occasional mitotic figures were found. Mitoses were also infrequent in the metastases to the lymph glands.

TABLE II

Classification of Developmental Stages

A. ADAMANTOBLASTOMA
   1. MONOCYSTIC
      (a) Monolobulated
      (b) Multilobulated
   2. MULTICYSTIC
      (a) Without solid epithelial cell masses
      (b) With solid epithelial cell masses
         (1) Squamous cell form
         (2) Epidermoid (with keratohyalin)
   3. SOLID ADAMANTOBLASTOMA
B. SARCO-ADAMANTOBLASTOMA

Developmental Stages of Adamantoblastomas

My classification of the developmental stages is based on the morphologic and histologic characteristics of these tumors (Tables II and III). The general term, adamantoblastoma, amply covers all central epithelial tumors which were found in these mandibles. The morphologic features are classified as cystic, solid, or both, and if cystic, as to whether monocystic or multicystic. The histologic features are further amplified by describing the characteristic epithelial form (Table II). It will be noted that in the classification of the multicystic types the squamous and epidermoid forms are emphasized. I purposely omitted from this classification other subclassifications such as adenomatous, stellate and ameloblastic epithelial forms, basal cell or embryonal types because the presence of such cells was infrequent in my tumors. This omission does not exclude the possibility of these subclassified forms attaining the prominence that the epidermoid form did in my group. In such event they become 2(b)(3), etc., in my classification.

1. *Monocystic Adamantoblastoma.* This type of epithelial tumor

(Fig. 39) is a growth composed almost entirely of a single cyst, devoid of epithelial masses, with an occasional exception. The monocystic multilobulated type includes those tumors with two or more connected cyst lobules.

2. *Multicystic Adamantoblastoma.* The main feature of these epithelial tumors are their multicystic character. They are further subdivided into two subheadings: (1) containing epithelial masses in the form of nests, strands, cords, or sheets (Figs. 40 and 44); and (2) those devoid of such cells (Fig. 41). Keratin and pearl formations may also be found in members of this group of epithelial tumors (Fig. 7).

3. *Solid Adamantoblastoma.* These growths are devoid of cysts. They are composed of solid epithelial masses containing more anaplastic features. Keratohyaline structures were found in all of my cases,

TABLE III

*Distribution of 103 Classified Adamantoblastomas**

	No. of animals	No. of tumors
Monocystic monolobulated	39	52
Monocystic multilobulated	4	4
Multicystic, without solid epithelial masses	10	13
Multicystic, with solid epithelial masses	7	9
Multicystic epidermoid	15	16
Solid adamantoblastomas	3	3
Sarco-adamantoblastomas	6	6

Other Tumors Observed

	No. of animals	No. of tumors
Odontomas	2	2
Sarcomas (pure)†	9	9
Ranula	1	..

* Seventy-two animals studied presented 114 tumors, of which 97 were pure adamantoblastomas, and 6 were sarco-adamantoblastomas.

† Of the 9 pure sarcomas, 5 were osteogenic, 2 spindle cell, 1 giant cell and 1 fibrosarcoma.

but such tumors without pearl formation would also fall in this classification (Fig. 42).

Sarco-adamantoblastomas

In six cases, sarcomas were found actively intermingled with the adamantoblastomas. This constitutes more than a rare finding and deserves to be classified separately (Figs. 22 and 46).

The foregoing classification, while based chiefly on the morphologic and histologic characteristics of these growths, may be further amplified through a consideration of both origin and malignancy. The origin, as has been pointed out, being the outer unspecialized epithelial cells of the enamel organ, makes possible the development of the diversified cell forms and modifications noted in my classification. In this respect

adamantoblastoma may be distinguished from "epitheliomas" which usually lack such diversification. It will be noted further that while the categorical arrangement of these growths depends upon their cystic or solid elements, there are also evident progressive degrees of malignancy, ranging from the benign purely cystic to the more malignant solid forms.

Discussion

Development of Adamantoblastomas

A considerable number of tumors could not be classified definitely in a single specific category but possessed characteristics of two or more types of adamantoblastomas. Thus, not all solid tumors were 100 per cent solid nor were all those cited as cystic tumors purely cystic. Several monolobulated tumors showed epithelial extensions from their cystic linings with cystic formations in them. Thus, a monolobulated cyst was being transformed into one which was multilobulated. However, these epithelial extensions do not always break down. In many cases the proliferation gives rise to solid masses of cells occupying considerable areas and as such transforms the tumor from the purely cystic to the cystic-solid variety (Figs. 43 and 45). Some of the cystic masses may break loose from their parent monocystic growth which thus becomes transformed into the multicystic variety. Thus, the monocystic tumors, if allowed to grow, in some instances retain their own individual features during the entire growth period while in others they take on characteristics of adamantoblastomas higher in the scale of malignancy.

Cahn [39] reported a single-cysted dentigerous tumor which contained mural swellings of epithelial cells in the cystic lining. This, he believed, had the potentiality of proliferating and giving rise to an adamantoblastoma. Churchill [37] and Schroff [38] reported follicular cysts with adamantoblastomatous characteristics. Hankey [17] described a multilocular cyst arising from what was thought to be a dental cyst. These cases in the literature tend to substantiate my observations of definite transformations in adamantoblastomas. This quality of transformation has been observed also in recurrences of human adamantoblastoma when second or third operations were necessary. Histologic study of the excised recurrent neoplasm frequently revealed more malignant features than the original. Lemaitre, Delater and Ardoin [51] described two such cases. In one instance the recurrence still presented a few adamantine structures but the typical cell had changed into a form resembling the squamous epidermal type. While previously it had seemed well encapsulated, the recurrence penetrated the surrounding tissues and assumed all the qualities of an infiltrating epithelioma.

The adamantoblastoma does not always exercise its transforming capabilities in the direction of more malignant forms. The opposite tendency has also been observed in my mice. Multicystic tumors were seen wherein the trabeculae separating individual cysts were being actively resorbed, thus simplifying their cystic structure. I also found one solid adamantoblastoma which showed evidences of early cystic degeneration (Fig. 10). Robinson [36] and Robinson and Wallace [70] reported such a case in a human being which degenerated from the solid to the cystic form. It is my opinion that if irritative factors such as surgical or other mechanical trauma and secondary infections were avoided, more adamantoblastomas would normally undergo cystic breakdown with dissolution of their solid epithelial masses. This does not mean, however, that all adamantoblastomas begin as solid epithelial tumors and become cystic only through breakdown.

The observed transformations from cystic to solid or from solid to cystic forms, with the many intermediate stages, raise a question as to the validity of segregating adamantoblastomas into the categories commonly used. It is probable that the different features are merely stages in the development and the life cycle of an adamantoblastoma. If it is observed at one stage, it is called a cystic adamantoblastoma; if it is observed at another stage, it is called a solid adamantoblastoma.

Epithelial Cell Types in Adamantoblastomas

The hypothesis based on the morphologic characteristics of the adamantoblastomas that the various forms constitute stages in development rather than independent types is not contradicted by the variety of epithelial cell types found in these growths. The outer embryonic layer of the enamel organ from which spring the cells comprising these tumors is capable of differentiating into many cellular forms. These may give rise to special features of the adamantoblastoma but need not necessarily alter the basic characteristic of the growth. The characteristic basal, squamous, or embryonal cells may not have prevailed as such throughout the entire life history of the growth, but may have been converted from one form into another.

Geschickter [27] stated that the more primitive elements of the enamel organ may proliferate and differentiate in several directions, producing islands of enameloblasts, squamous cells and basal cells, a mixture of epithelial elements characteristic of adamantoblastoma. Gioia [71] described a cystic adamantoblastoma lined by columnar epithelium. Moore [53] classified these tumors by cell type as cuboidal, columnar and stellate. Lemaitre, Delater and Ardoin [51] described two cases of adamantoblastoma in which the typical cell was squamous epidermal in

solid sheets and with some keratinization. Adcock [72] presented a case of adamantoblastoma which was of the glandular cystic type. Sprawson and Keizer [61] described structures in such tumors which simulate the stellate reticulum of the enamel organ. Gentsch [26] stated that all three types of enamel organ cells are represented in an adamantoblastoma; *i.e.*, the outer, flattened cuboidal cells, the intermediate layer of polygonal cells and the high cylindrical palisade cells which are the true ameloblasts. In the adamantoblastomas observed by these authors the various epithelial components are natural consequences of pluripotential embryonic origin with the possibility of giving rise to essentially all types of epithelial cells during the proliferative state. Ewing [15] maintained that these tumors are mainly of one nature and that the epithelial cells of adamantoblastomas, in exhibiting all variations in form between the stratified squamous epithelium and specialized adult enameloblasts, recall the changes traversed in the normal development of the enamel organ. Thoma,[73] too, stated that adamantoblastomas may present a multitude of epithelial cell forms. He recognized such types as basal cell, adenoid, acanthoma and keratinizing.

Adenomatous structures observed in my adamantoblastomas never assumed the rôle of typical cells for any one tumor. Oral epithelium has the potential ability to form glandular as well as dental structures and, since the components of the enamel organ are derived from the epithelium of the oral cavity early in fetal life, the embryonic potentiality of giving rise to cells which may differentiate into adenoma-like structures may exist. The more specialized columnar epithelial cells, some well differentiated and others more anaplastic, give further evidence of the multipotential powers of differentiation of the outer epithelial cells of the enamel organ. The more frequent occurrence of the squamous cell type may be explained on the basis that the embryonic cells composing the outer layer of the enamel organ should tend to reproduce in a manner similar to their immediate parent cells, the oral epithelium.

Stroma

The various forms of stroma tend to portray the proliferative powers and malignant tendencies of the adamantoblastomas. For example, it was observed that richly cellular matrices and sarcomatous stromas were found in the more active-appearing tumors, in which infiltration and invasion of neighboring structures by long extensions of epithelial cells were prominent features. The solid adamantoblastomas invariably contained areas of fibroblastic stroma. Bauer [14] also recognized the significance of the connective tissue stroma. He noted that in the cystic types there is usually an insignificant increase of the connective tissue,

while in some of the more solid tumors the stroma is greatly increased and plays an important rôle.

The cystic forms of adamantoblastoma, types known to be slower growing and less active than those with large areas of solid epithelium, usually contained stroma composed largely of hyalinized connective tissue bundles, comparatively acellular. The picture here contrasted markedly with those tumors in which the stromal and epithelial proliferation gave definite signs of malignancy. The cellular quality of the connective tissue stroma varied directly with the epithelial proliferation in the solid portions of adamantoblastomas. Probably because of desmoplastic properties of the growths, the stroma of some of them is so activated that the connective tissue assumes sarcomatous features, or definite mixed tumors—sarco-adamantoblastomas—may develop as I have found in this series.

Formation of Enamel

Although it is frequently stated in the literature, I have never observed tumor-formed enamel in any of my adamantoblastomas. Kronfeld [5] and Robinson [7] stated that enamel is never formed in adamantoblastomas. Churchill [37] never observed amelogenesis in true adamantoblastomas. He stated that enamel formation is possible only when there is an arrangement of the differentiated cells similar to that present during the formation of a tooth. I observed enameloblasts as well as cells of the stellate reticulum and stratum intermedium in the solid epithelial portions of these tumors in mice, but evidence of functional activity was never seen. The lack of tumor-formed enamel in pure adamantoblastomas is to be expected because enamel is deposited only when it has been preceded by, and is in the presence of, already formed dentin or a similar structure. Since dentin, which is of connective tissue origin, is not formed in an epithelial tumor such as the adamantoblastoma, the formation of enamel, dependent on the presence of dentin, must be and is precluded.

However, two growths were found which did contain tumor-formed enamel in conjunction with tumor-formed dentin (Figs. 47 and 48) arranged with wild, disordered patterns. Many individual neoplastic enamel organs of various sizes and shapes were seen, with the active production of enamel about previously formed dentin. There were proliferations of dentinoblasts within the crescent-shaped enamel organs and in many instances pre-dentin had been deposited but enamel formation had not yet begun. These two tumors, showing neoplasia of both epithelial and connective tissue, were true *odontomas* and should not be confused with the pure epithelial adamantoblastomas.

Malignancy

Adamantoblastomas are not benign growths if we accept Ewing's [15] criteria for malignancy. We find that adamantoblastomas do not remain localized and circumscribed. They exhibit features which are diametrically opposed in character to benign growths. The adamantoblastoma is an infiltrative growth. Its epithelial columns and strands invade surrounding bone marrow spaces, the ramus of the mandible, the masseteric muscles, the base of the tongue and even spread to the opposite half of the mandible. This tumor possesses the power of destroying regional tissues. Resorption of bone takes place over extensive areas by the massive cystic expansions and pressure of proliferating epithelial sheets.

While my investigation of these tumors of mice could not include a study of the possibility of recurrent growths after removal, Robinson,[7] in a review of 379 cases of human adamantoblastomas, recorded 119 in

TABLE IV

	Mice (97 adamantoblastomas found in the group of mice)		Man (397 cases in Robinson's [7] review of the literature)	
	Number	*Percentage*	*Number*	*Percentage*
Invasive growths or recurrent growths	30	30.9	119	31.4
Metastatic growths	2	2.1	9	2.4

which there were from one to as many as twenty-two recurrences. Recurrences are possible because of the invasive characteristics, negating the likelihood of complete surgical removal. There are cases on record where complete resection of a mandible was followed by recurrence. Benign growths do not exhibit these invasive qualities nor the same tendency to recur. If we consider the degree of infiltration and invasion in my animals, we find that similar recurrence would have been possible. Thus, out of a total of 97 pure adamantoblastomas, 30, or 30.9 per cent, exhibited the quality of invasion into the surrounding tissues (Table IV). This figure compares favorably with Robinson's 119 recurrences in 379 cases (31.4 per cent) and emphasizes the idea that the infiltrating quality of these tumors predisposes to recurrences.

Robinson [7] recorded 9 metastasizing tumors in 379 cases reported in the literature (2.4 per cent). This compares favorably with my 2 cases with metastases, which is 2.1 per cent of my series.

However, Stout [16] maintained that for practical purposes the probability that any given adamantoblastoma will metastasize is negligible. While this statement is borne out in my series, nevertheless the fact that metastases do occur furnishes further evidence against considering

these growths benign. Robinson [7] considered these tumors as persistent but not malignant. It must be pointed out that persistency is a trait of malignant tumors.

I have observed in my experimental group tumors duplicating in all respects the extensive variety of human adamantoblastomas. They are similar in morphology, cellular constituents, destructive actions, invasions and metastases. In many respects they are identical. Thus, that which applies to my experimental group may be said to hold for this tumor in man. From my observations, adamantoblastomas must be considered not as purely benign tumors, but as *locally malignant tumors* with a slight potentiality to metastasize.

Origin

In considering the site of origin of adamantoblastomas, those reported in the literature are based, of necessity, on limited observations because of the surgical nature of the human material studied and the embryologic difficulties involved. In spite of these limitations certain definite ideas prevail, the most common one being that the epithelial rests are the site of origin. Since the time of Malassez' original observation of the presence of these rests in the peridental membrane of teeth, there has been a steady stream of reports in the literature pointing to these rests as the source of origin of adamantoblastomas. Malassez himself did not report actual observations of tumors arising from epithelial cell nests. He merely assumed that these epithelial structures might proliferate and give rise to tumors. Yet, many authors who followed took it for granted that these rests were the actual source of the growths which they reported and so stated in their reports.

In my study I was especially apprehensive concerning this possibility, searching carefully for any sign of proliferation of the epithelial rests or even contiguous location of an adamantoblastoma to one of them. In most of my cases, epithelial rests were not found, and in those few cases where they were observed they were conspicuously inactive. Not a single adamantoblastoma was observed contiguous to these rests in the peridental membrane. In fact, nearly all of my tumors were actually in a position diametrically opposed to the location of epithelial rests. In no possible way could they have originated from these structures in the peridental membrane. From these observations, we may definitely rule out the epithelial rests of Malassez as a source of origin of adamantoblastomas in this group of mice.

A little more weight may be given to the observations citing the oral epithelium as the source of origin. Von Bakay,[32] Kuru,[33] Kaufmann [34] and, more recently, Cahn [74] reported cases of this type. In my own

series of animals I observed eight cases in which there were definite attachments of the adamantoblastomas to the oral epithelium. But these eight tumors had other points of attachment, *i.e.*, to the outer epithelium of the enamel organ. It is more probable that this latter point of attachment was the true site of origin since similar tumors in this series were so attached without being continuous with the oral epithelium. Attachment to the oral epithelium is apparently a secondary manifestation caused by traumatic or irritative ulceration of the oral surfaces, thereby providing an opportunity for stimulating the proliferation and downgrowth of the oral epithelium which in turn becomes attached to the adamantoblastoma. In the cases reported in the literature, only portions of the adamantoblastomas could be examined, hence the possibility of another site of attachment in those cases could not be ascertained. These observations, then, cannot compare with mine where it was possible to study the whole growth ***in situ***.

The possibility of these growths arising from the epithelium of odontogenic cysts is in accord with my own findings. However, since it is my opinion that odontogenic cysts are merely stages in the development of adamantoblastomas, the proliferation of their epithelial linings may be considered as extensions or modes of transformations rather than as sources of origin. This study provides ample evidence to show that the outer embryonal epithelium of the enamel organ is the source of origin of these growths and may be considered as the only source of origin of the adamantoblastomas found in my animals.

"Adamantoblastomas" of Other Parts of the Body

Geschickter [27] stated that the occurrence of adamantoblastomas in the hypophyseal stalk and in the tibia is against the specificity of the enamel organ as a source for these growths. This idea would apply to the ameloblasts, cells of the stratum intermedium and the stellate reticulum as sources of origin. These specialized cells of the enamel organ would have to undergo metaplasia in order to form tumors such as I found. However, I do not believe that adamantoblastomas are due to metaplasia of such cells. The fact that tumors simulating the adamantoblastoma of the jaw have been found in the hypophyseal stalk and in the tibia does not detract in any way from my views as to origin, because of the embryonal character of the fourth layer of the enamel organ which I have designated as the source of the adamantoblastoma in jaws.

That the pituitary duct arises as an invagination of the oral epithelium amply explains the possible similarity of the two types of epi-

thelial tumors under discussion. They both maintain their embryonic character, and both may differentiate into a variety of epithelial cell forms, as stated in the literature. It should be borne in mind that neither in the adamantoblastoma of the jaws nor in the adamantoblastoma of the hypophysis are formed tooth elements found. The few adamantoblastomas found in the tibia likewise do not contain formed tooth elements and their presence may also be explained on the basis of abnormal embryonic epithelial invaginations. Thoma,[8] Drummond,[46] Ryrie [75] and Oberling, Vermes and Chevereau [76] concur in this view. Whether these cells of origin represent isolated structures such as cell rests or epithelial implants, or whether they comprise a portion of a known anatomic formation such as the hypophyseal stalk, craniopharyngeal duct, or the enamel organ is not of essential importance. What is important is that they arise from similar epithelial parents, *i.e.*, stratified squamous epithelial structures, and that they retain similar embryonic qualities and multipotential powers in their growth tendencies.

Sarco-adamantoblastomas

There is no doubt that the sarco-adamantoblastoma is a distinct entity. These mixed tumors contain the morphologic and histologic structure of the sarcoma and the adamantoblastoma. Whether the sarcomatous features develop as a result of a desmoplastic action by the adamantoblastoma and are therefore secondary, whether the reverse is true, or, whether both begin simultaneously I cannot support with indisputable microscopic evidence. I am inclined to believe, however, that the sarcomatous manifestations are secondary to the original adamantoblastoma since I found epithelial tumors with sarcoma-like stroma, probably representing an intermediate stage. In several instances it was difficult to differentiate adamantoblastoma with a sarcomatous stroma from sarco-adamantoblastoma.

Are Dentigerous, Follicular and Multilocular Cysts Forms of Adamantoblastoma?

Although some authors believe these to be distinct entities, in no way related to adamantoblastomas, it is my opinion that dentigerous, follicular and multilocular cysts are stages of adamantoblastomas and comparable to the pure monocystic and multicystic stages of tumors found in my mice. In them, simple cystic growths present the same morphologic and histologic features and may or may not envelop the crown of a tooth (Figs. 43 and 49). The origin in the outer epithelial layer of the enamel organ is the same for these purely cystic tumors as

for those higher in the scale of malignancy. It has been noted that the epithelial linings of dentigerous cysts may proliferate and lead to adamantoblastomatous growths.

Conclusions

One hundred and three adamantoblastomas, found in a group of 79 mice with hereditary tumor tendencies obtained from the Slye colony at the University of Chicago, served as a basis for this report. In each instance the animal was allowed to live its normal life span. These adamantoblastomas were cystic, solid, or both cystic and solid.

From observations upon this group of adamantoblastomas it is concluded that:

1. Odontogenic cysts are stages in development of the adamantoblastoma.
2. Types of pure adamantoblastomas are not independent varieties but must be considered as stages in the development of these growths.
3. Enamel is never formed in pure adamantoblastomas.
4. The assertion that "adamantoblastomas" are found in other parts of the body in no way refutes the conclusion that adamantoblastomas of the jaw arise from the enamel organ of a tooth.
5. The point of origin of adamantoblastomas in the jaw is the group of embryonal cells comprising the outer epithelial layer of the enamel organ.
6. Adamantoblastomas are not characterized by the features usually ascribed to benign growths. They do, however, possess either one or more of the criteria for malignancy. Therefore, they should be designated as locally malignant with a slight potentiality to metastasize.

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DESCRIPTION OF PLATES

PLATE 4

FIG. 1. Lateral radiographic view illustrating extensive involvement of bilateral tumors. Mouse 20. (A) Area of previous excision for biopsy. (B) Large cystic portion of tumor outlined by arrows. (C) Tremendous elongation of incisor teeth due to malocclusion; these tend to form circular structures by their continuous growth. (Normal mandibular outlines are obliterated by the tumors which cause bone resorption. The ramus in this view has been destroyed.) × 4.

FIG. 2. Ventral radiographic view illustrating bilateral cystic adamantoblastomas. Mouse 36. (A) Monocystic multilobulated adamantoblastoma with bone septa clearly distinguishable. (B) Displacement of lower incisor teeth by expansive tumc growth. (C) Elongation and malocclusion of incisor teeth. (D) Monocystic adamantoblastoma. (Note the outward expansiveness of the growth.) Tumor diagnoses were made following histologic study. × 4.

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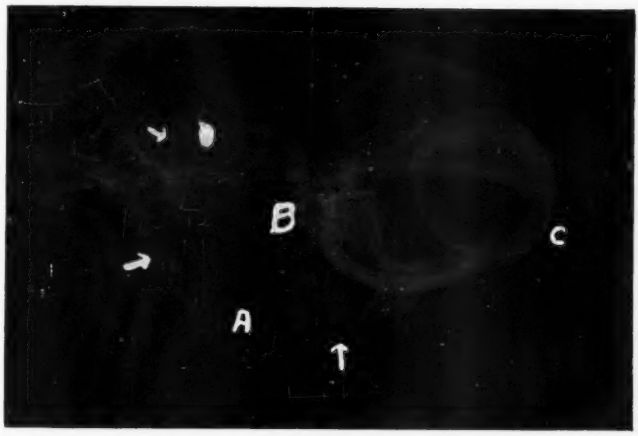

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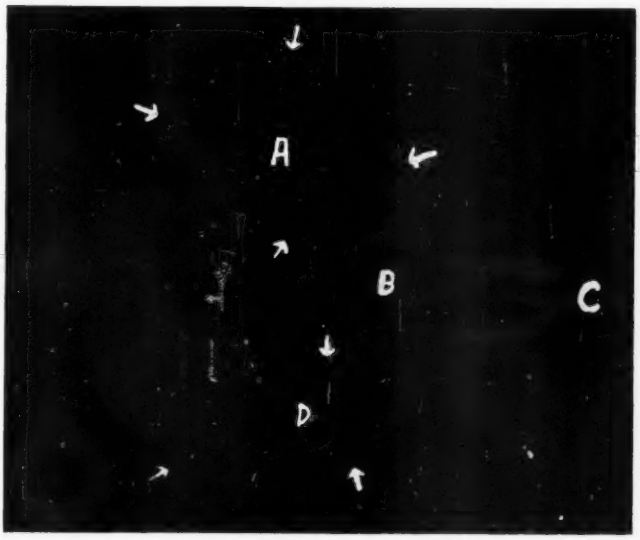

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Plate 5

Fig. 3. Cross section (all photomicrographs are cross sections unless otherwise stated) showing normal structures of the mandible. Mouse 7. (A) Molar tooth. (B) Body of mandible. (C) Mandibular canal. (D) Cross section of unerupted portion of incisor tooth. (E) Peridental membrane. (F) Dentin. (G) Pulp of tooth. (H) Enamel space. (I) Enamel organ. (J) Tongue. (K) Oral mucous membrane. × 22.

Fig. 4. Marked distortion of tooth contour. Mouse 20. (A) Marked distortion of cross section of incisor tooth simulating a longitudinal section. (B) Irregularly shaped and placed enamel space. (C) Multicystic solid adamantoblastoma. (D) Lengthening and atrophy of striated cheek muscles. (E) New bone formation surrounding the tumor. (F) Inflammatory masses in cysts. (Note the loss of normal bone outlines when compared to Fig. 3.) × 12.

Fig. 5. Cystic characteristics. Mouse 31. (A) Stratified squamous epithelium lining the cyst. (B) Desquamated epithelial cells. (C) Cholesterol slits. (D) Inflammatory masses. (E) Hemorrhagic débris. (F) Foam cells. (G) Artefact. (H) Enamel space. (I) Dentin. (J) Stroma. × 160.

Fig. 6. Cystic characteristics. Mouse 10. Ramus region. Multicystic adamantoblastoma showing (A) multicystic character. (B) Bone septa. (C) Stratified squamous epithelium lining the cysts. (D) Clear cystic fluid. (E) Inflammatory cystic exudate. (F) Displaced incisor tooth. (G) Destruction of ramus by the invasion of neoplastic tissue. (H) New bone formation at periphery of cysts. (I) Lengthening and stretching of striated muscles. (Note loss of normal bone outlines.) × 11.

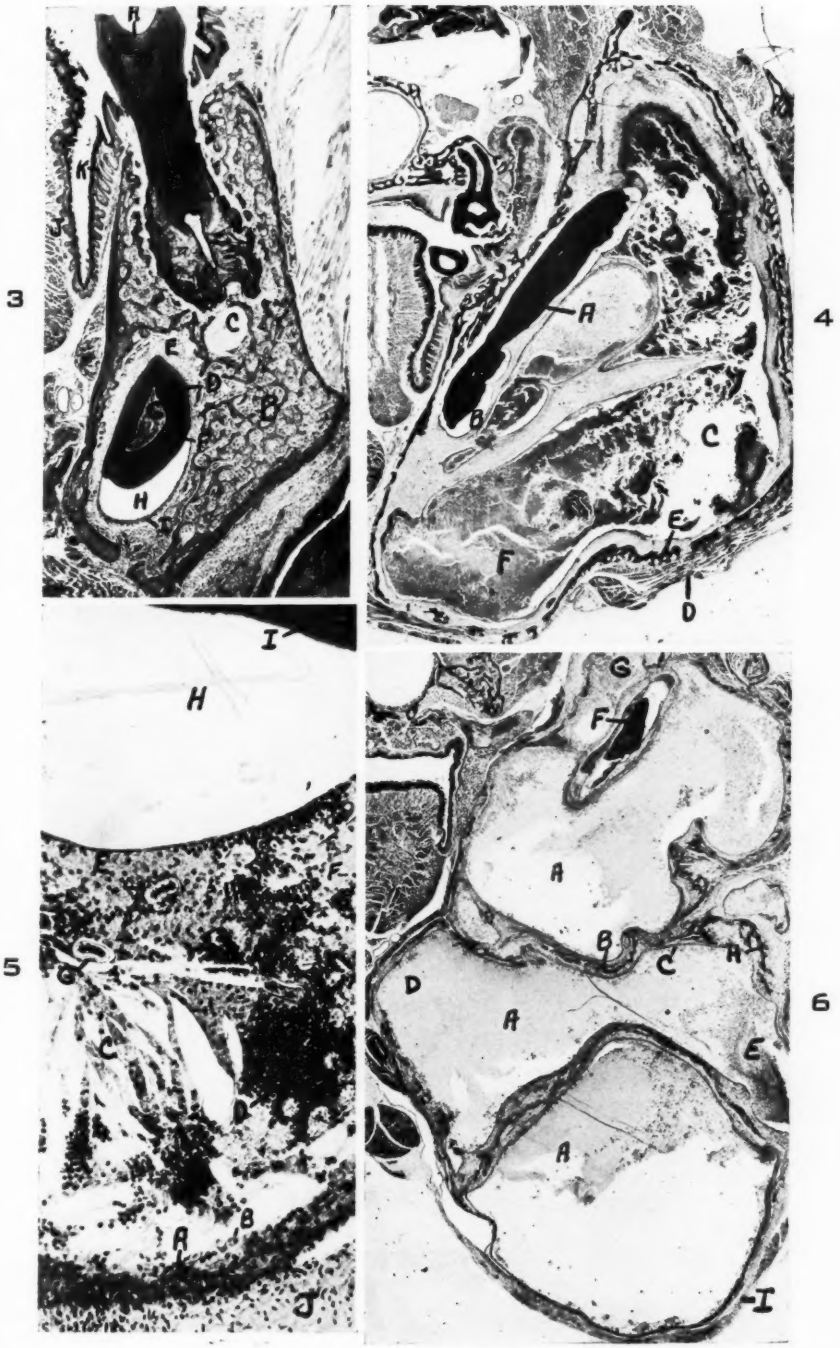

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PLATE 6

FIG. 7. Longitudinal section of multicystic epidermoid adamantoblastoma. Mouse 62. (A) Keratohyaline structures, some in the process of dissolution with the degenerative masses tending to form cysts. (B) Region of previous excision for biopsy. (C) Epithelial sheets and cords, branching and anastomosing. (D) Attachment to the enamel organ. (Note: The tumor is below and posterior to the enamel space, "E.") × 13.

FIG. 8. Longitudinal section showing growth below and posterior to the enamel space and tooth. Mouse 43. (A) Attachment to the enamel organ. (B) Multicystic solid tumor. (C) Enamel space. (D) Incisor tooth. (E) Peridental membrane. (F) Tongue. × 10.

FIG. 9. Cystic characteristics. Mouse 10. (A) Cyst lining composed of flattened, lengthened stratified squamous cells. (B) Desquamated epithelial cells. (C) Inflammatory cells. (D) Hemorrhage. (E) Foam cells. (F) Bone septa with (G) fibrous connective tissue stroma. × 350.

FIG. 10. Keratohyaline structures. Mouse 51. (A) Epithelial pearls. (B) Beginning cystic breakdown. × 155.

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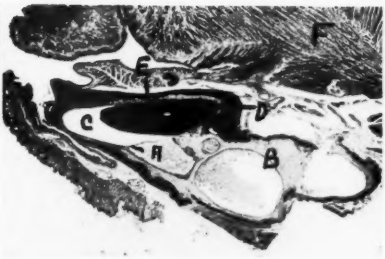

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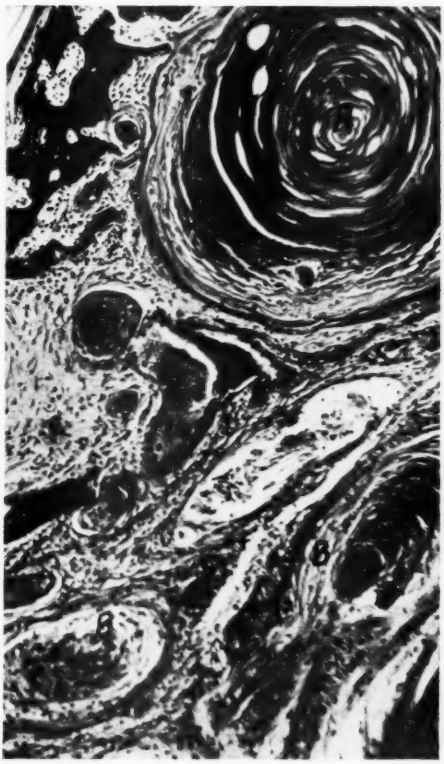

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Adamantoblastomas in the Slye Stock of Mice

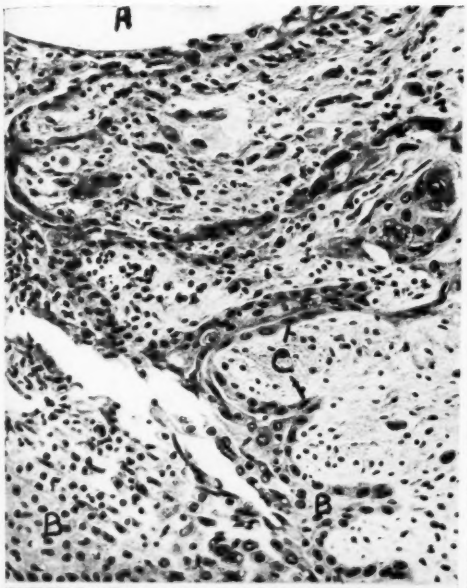
PLATE 7

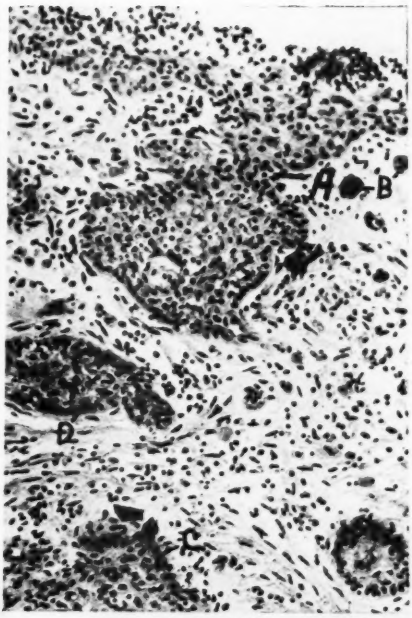
FIG. 11. Solid epithelial characteristics. Mouse 13. (A) Enamel space. (B) Stratified squamous epithelium with intercellular bridges. (C) Epithelial extensions and cords permeating the surrounding stroma. × 255.

FIG. 12. Solid epithelial characteristics. Mouse 9. (A) Epithelial extension from cystic lining. (B) Cross sections of epithelial cords. (C) Undifferentiated-appearing epithelial masses. (D) Alveolar or stellate arrangement. × 195.

FIG. 13. Solid epithelial characteristics. Mouse 19. (A) Adenoma-like epithelial cells. (B) Hyperchromatic nuclei. (C) Bone spicules in process of resorption. (D) Osteoclast in Howship's lacunae. (E) Central cystic breakdown of solid epithelial portions. × 255.

FIG. 14. Solid epithelial characteristics. Mouse 57. (A) Undifferentiated basal-like epithelial cells, carcinomatous in appearance. (B) Bone spicules in process of resorption. (C) Hyperchromatic nuclei. (D) Epithelial mass undergoing cystic breakdown. × 145.





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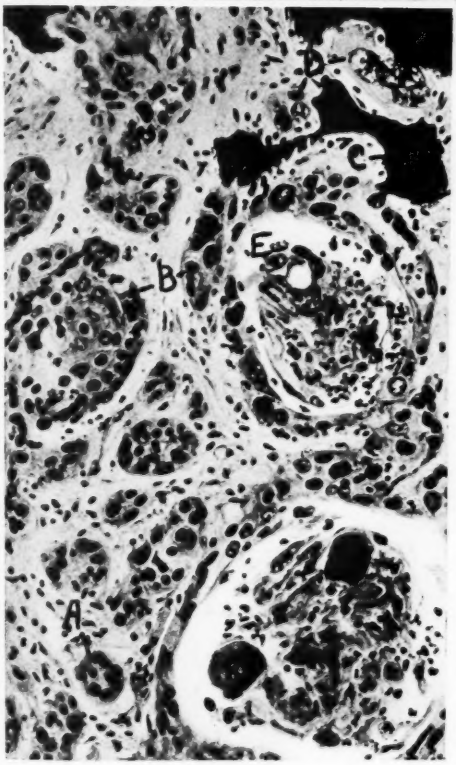

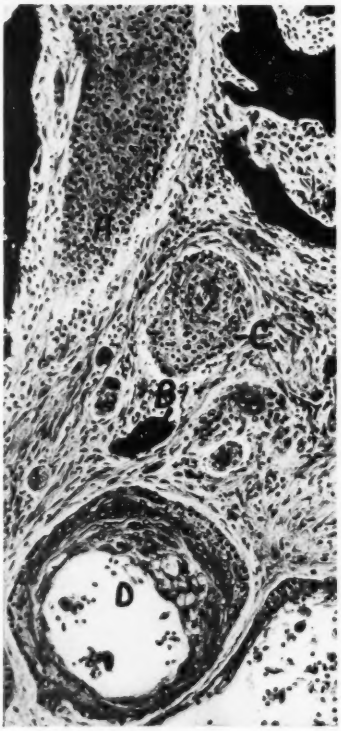

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Plate 8

Fig. 15. Solid epithelial characteristics. Mouse 16. (A) Embryonal epithelial syncytium with anaplastic features. (B) Cross-section of epithelial strand simulating a giant cell. (C) Foreign body giant cell. (D) Stroma. (There is very little stroma present.) × 395.

Fig. 16. Epithelial pattern simulating spindle cell sarcoma. Mouse 38. × 590.

Fig. 17. Hyalinized collagenous stroma. Mouse 20. (A) Hyalinized stroma of a multicystic solid adamantoblastoma. (B) Hemorrhagic cystic content. (C) Displaced portions of incisor tooth. (D) New bone formation at periphery of tumor. (Note expansiveness of tumor with consequent effect on masseter muscles.) × 12.

Fig. 18. High-power photomicrograph of Figure 17. (A) Hyalinized stroma. (B) Cystic lining. (Vascularity is minimal.) × 460.

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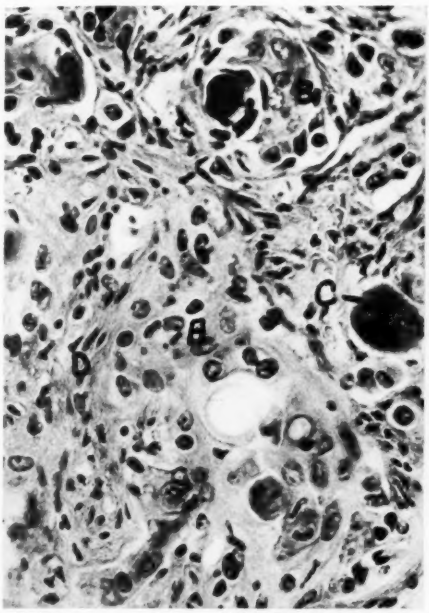

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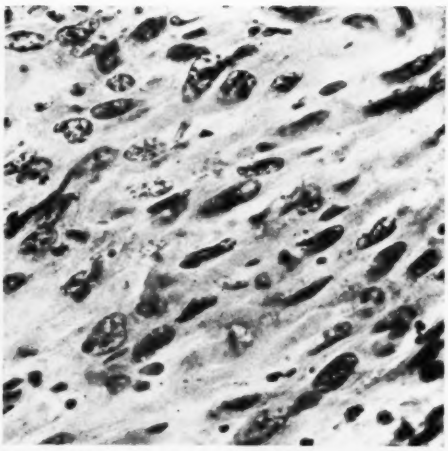

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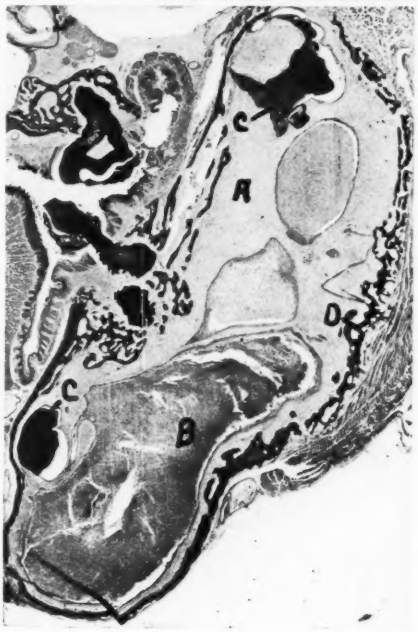

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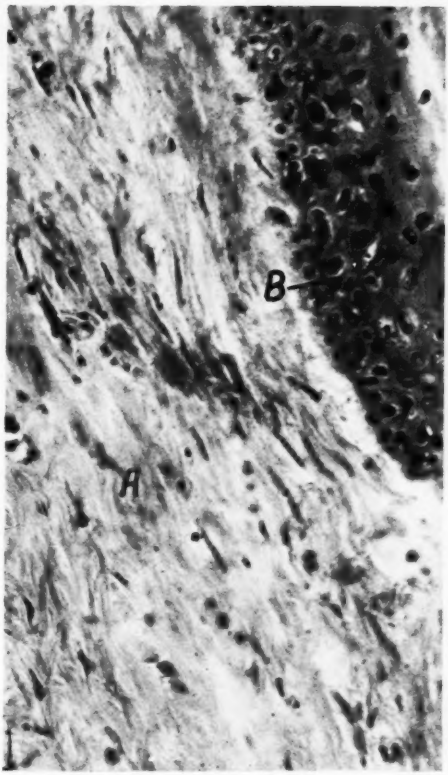

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Adamantoblastomas in the Slye Stock of Mice

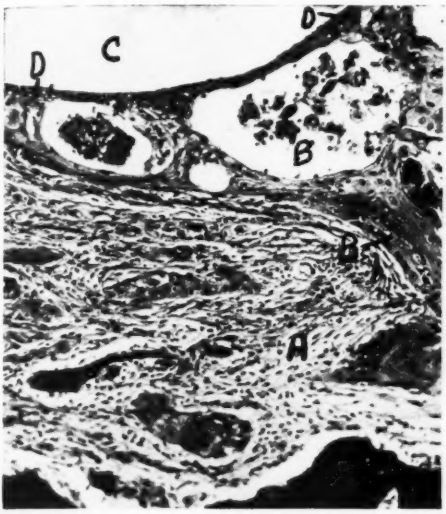
PLATE 9

FIG. 19. Cellular stroma. Mouse 59. (A) Cellular stroma. (B) Stratified squamous epithelial strands with cystic breakdown. (C) Enamel space. (D) Attachment of tumor to enamel organ. × 160.

FIG. 20. Sarcomatous stroma. Mouse 38. (A) Sarcoma-like stroma. (B) Infiltrating epithelial strands. × 280.

FIG. 21. Rich vascularity simulating an hemangioma. Mouse 38. (A) Rich capillary networks. (B) Fibrocellular stroma. (C) Enamel organ with outer embryonal epithelial cells at "D." (E) Hemosiderin granules. × 192.

FIG. 22. Desmoplastic characteristic. Sarco-adamantoblastoma. Mouse 17. (A) Epithelial proliferation from outer layer of cells of enamel organ. (B) Spindle cell sarcoma. (C) Enamel space. (D) Dentin. × 134.



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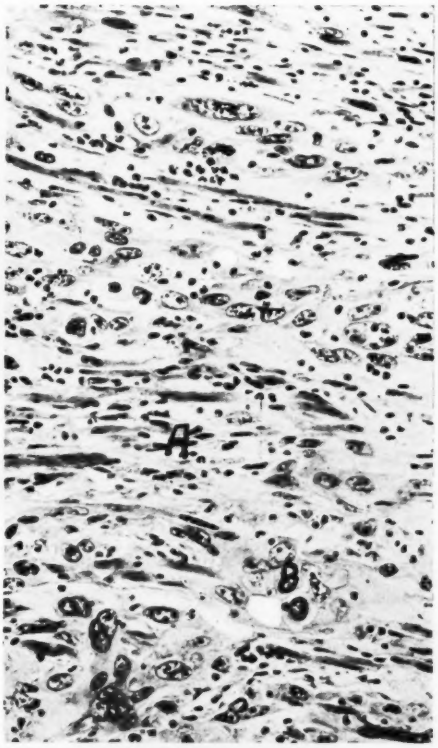

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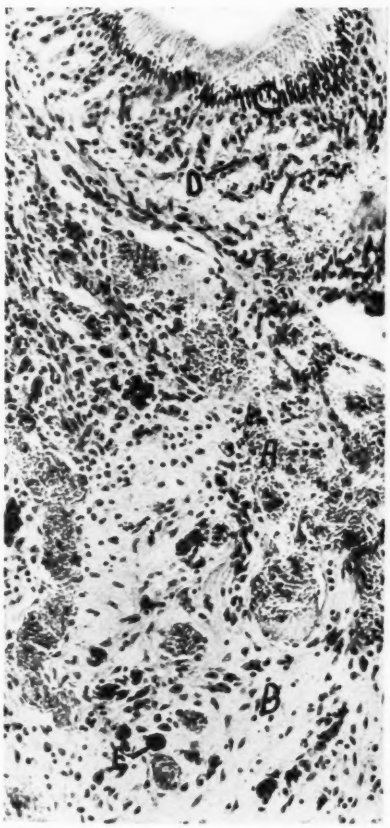

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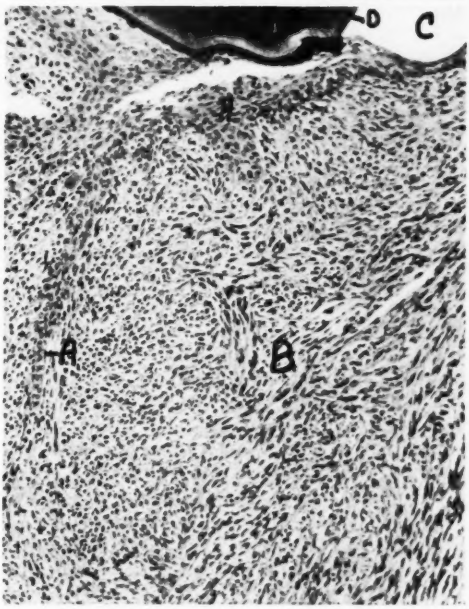

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Adamantoblastomas in the Slye Stock of Mice

PLATE 10

FIG. 23. Epithelial rest in peridental membrane with proliferation from the enamel organ forming an adamantoblastoma. Mouse 12. (A) Inactive epithelial rest in peridental membrane on the concave side of the tooth. (B) Proliferative mass from outer layer of cells of the enamel organ, on the convex side of the tooth. (C) Enamel space. (D) Dentin. × 105.

FIG. 24. High-power photomicrograph of Figure 23. (A) Inactive epithelial rest in peridental membrane of a tooth of which the enamel organ gave rise to an adamantoblastoma. (B) Peridental membrane fibers. (C) Dentin. × 1315.

FIG. 25. Developing normal incisor. Mouse 10. (A) Outer layer of embryonal epithelial cells of the enamel organ which are considered the origin of adamantoblastomas. (B) Enameloblasts. (C) Decalcified remnant of enamel. (D) Dentin with dentinal tubules. (E) Odontoblasts of pulp. (F) Shrinkage artefact. × 460.

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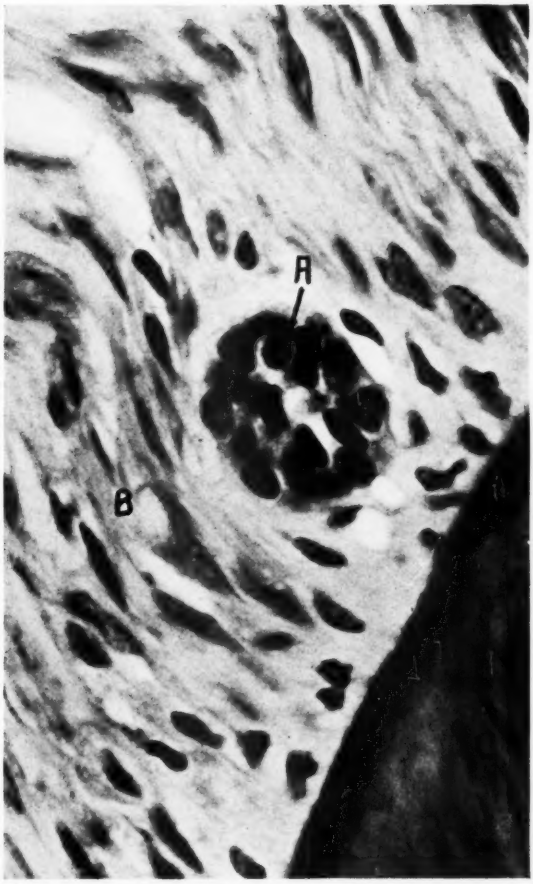

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PLATE 11

FIG. 26. Origin of adamantoblastomas. Mouse 30. (A) Proliferation of the outer epithelium of the enamel organ forming an adamantoblastoma. (B) Enamel space. (C) Dentin. × 183.

FIG. 27. Origin of adamantoblastomas. From the same animal as shown in Figure 26 but at a more posterior level, showing a more advanced stage of development. (A) Beginning cystic breakdown. × 240.

FIG. 28. Origin of adamantoblastomas. Mouse 36. (A) Epithelial proliferations arising from the enamel organ at several points. (B) Early stage in the development of a simple monocystic adamantoblastoma with attachments to the enamel organ. (C) Enamel space. (D) Cystic space. (Proliferations are below the enamel space, on the convex side of the tooth.) × 15.

FIG. 29. Origin of adamantoblastomas. Mouse 10. (A) Extremely broad surface attachment where almost the entire outer layer of enamel organ is involved as the site of origin. × 240.

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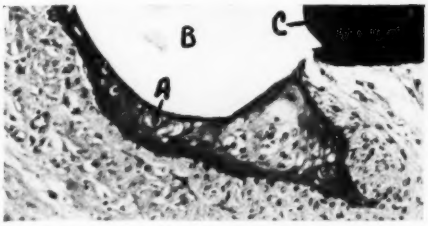

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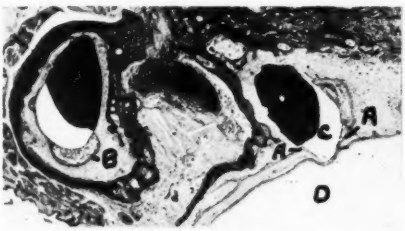

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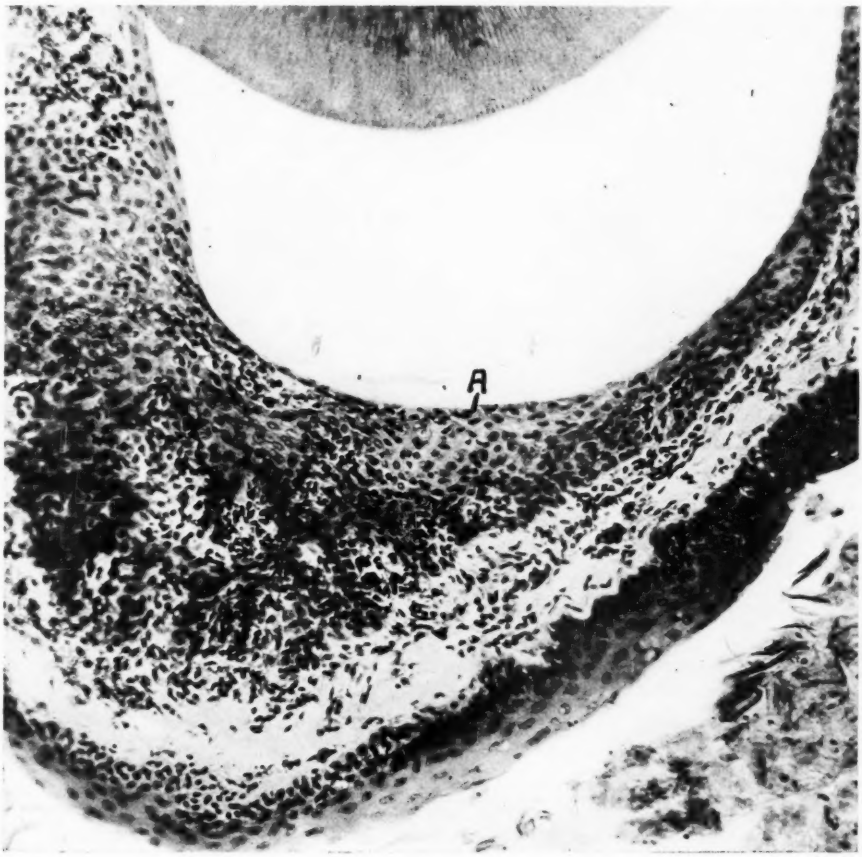

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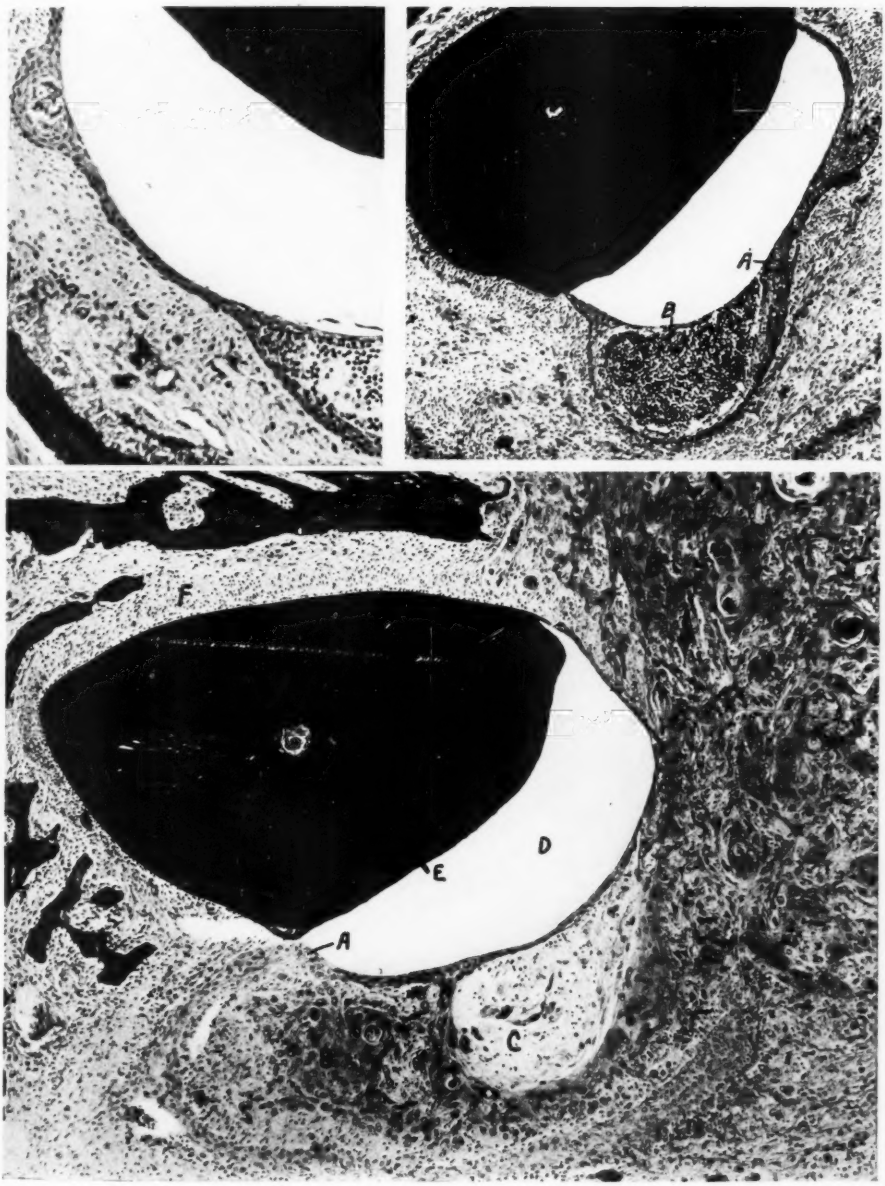
PLATE 12

FIG. 30. Origin of adamantoblastomas. Mouse 36. Multiple sites of origin from the outer layer of the enamel organ. × 150.

FIG. 31. Origin of adamantoblastomas. Mouse 35. (A) Attachment of tumor of the enamel organ outside the enamel space. (B) Inflammatory exudate in cyst. × 85.

FIG. 32. Origin of adamantoblastomas: carcinomatous. Mouse 38. (A) Attachment at point of origin to the enamel organ. (B) Squamous cell carcinomatous character of the tumor. (C) Beginning cystic breakdown of epithelial masses. (D) Enamel space. (E) Dentin. (F) Peridental membrane without involvement. × 105.



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Adamantoblastomas in the Slye Stock of Mice

PLATE 13

FIG. 33. Metastasis in a lymph node. Mouse 38. (A) Compressed lymphoid structures. (B) Metastatic multicystic epidermoid adamantoblastoma. (C) Cystic breakdown in the metastasis. × 36.

FIG. 34. Metastasis in a lymph node. High-power view of Figure 33. (The more anaplastic-appearing epithelial cells should be compared with Figure 16 which is the parent tumor.) × 135.

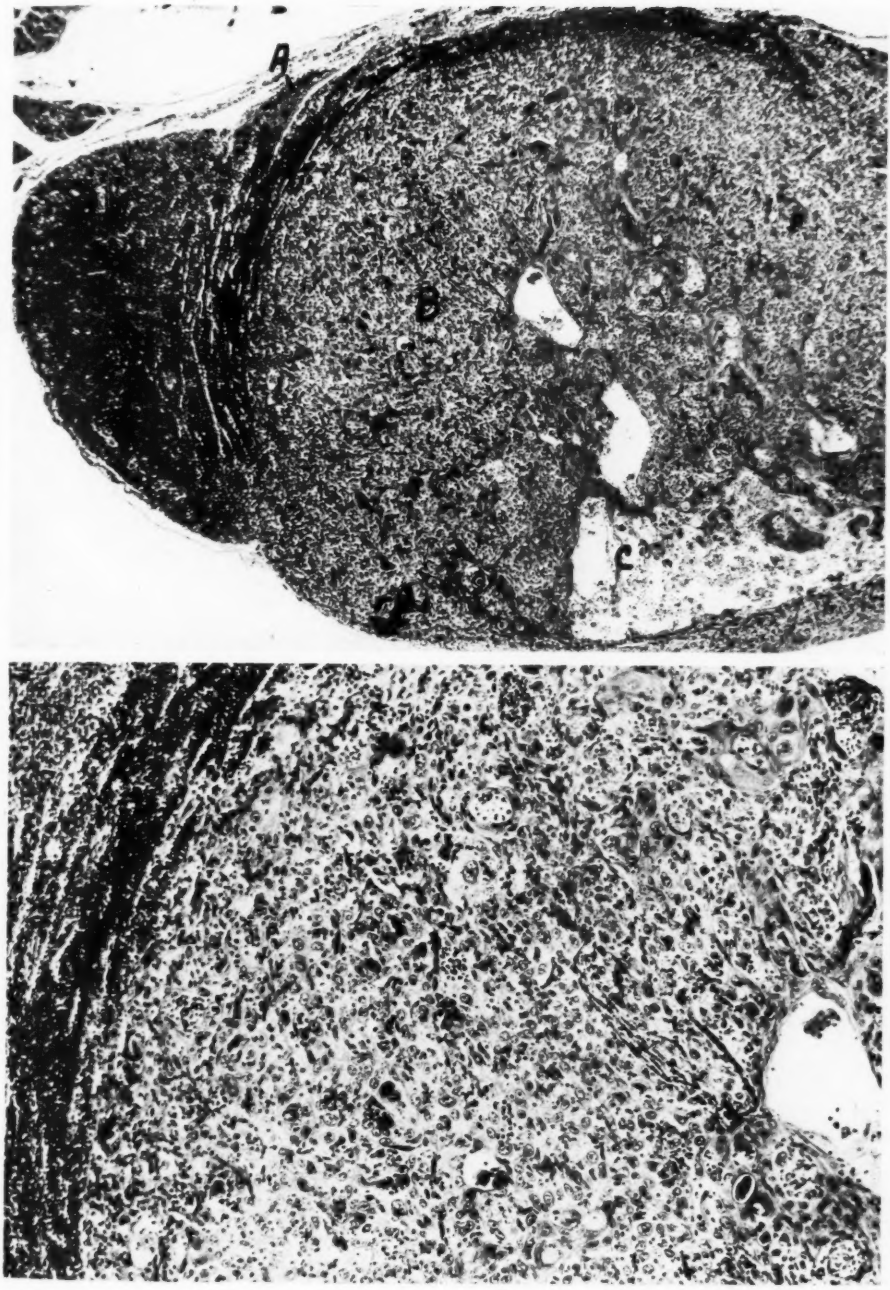

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Plate 14

Fig. 35. Invasion of tumor into surrounding bone. Mouse 40. (A) Epithelial tumor strands from a solid epidermoid adamantoblastoma invading surrounding bone structures. (B) Keratohyaline formations. (C) Evidences of very early cystic breakdown of epithelial masses. × 175.

Fig. 36. Invasion of tumor masses into bone spaces. Mouse 72. There are wild, bizarre epithelial patterns. × 145.

Fig. 37. Infiltration of tumor into muscle. Mouse 57. × 208.

Fig. 38. Mitotic figures. Mouse 57. Epithelial strands of a multicystic epidermoid adamantoblastoma with cells in various phases of mitosis. × 1260.

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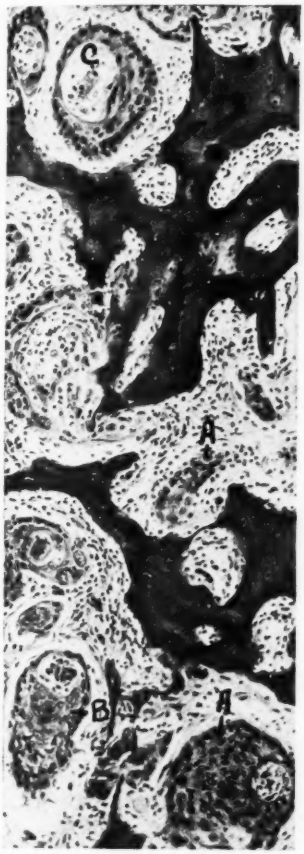

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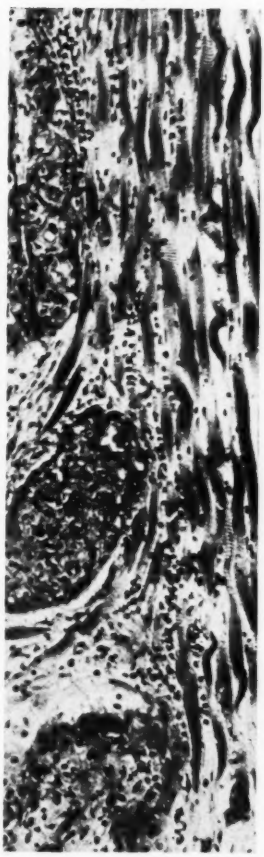

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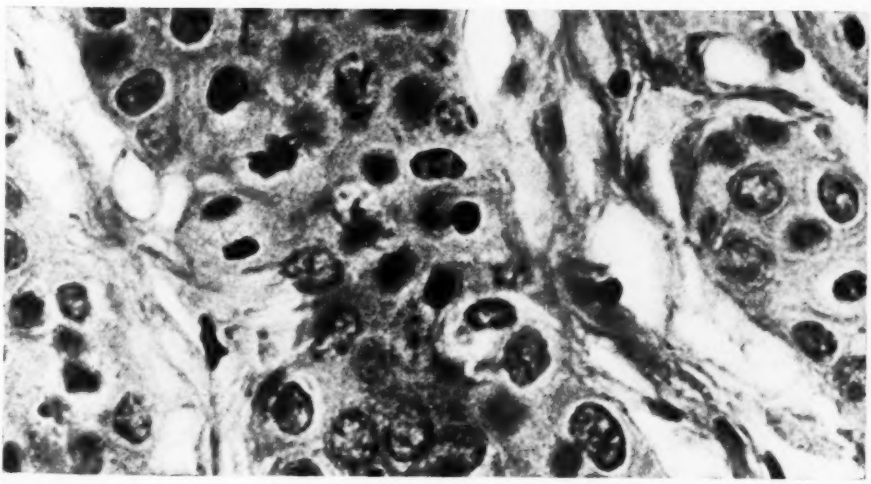

PLATE 15

FIG. 39. Stages in development of adamantoblastoma. Mouse 2. (A) Monocystic adamantoblastoma with no solid epithelial proliferations and with a minimal amount of stroma. (B) Displaced incisor tooth from whose enamel organ this tumor arose. × 8.

FIG. 40. Stages in development of adamantoblastomas. Mouse 2. (A) Multicystic adamantoblastoma. (B) Small amount of inflammatory exudate in cyst. (C) Hemorrhagic cystic content. (D) Tongue. (E) Stretching and lengthening of masseter muscles by expansive growth. (F) Resorption of ramus and replacement by neoplastic tissue. (This tumor is one of bilateral growths in a single animal. Figure 39 represents the other tumor. See also Figure 6 for another multicystic adamantoblastoma.)

FIG. 41. Stages in development of adamantoblastomas. Mouse 13. Multicystic solid adamantoblastoma. (A) Cystic portions. (B) Solid epithelial areas with numerous small areas of cystic breakdown among epithelial masses and strands. (C) Enamel space surrounded at "D" by enamel organ proliferations. (E) Incisor tooth displaced lingually by tumor growth. (F) Posterior root portion of same tooth which has been displaced superiorly by tumor. (G) Oral ulceration but with no epithelial proliferation from this source. (Note thinness and lengthening of masseter muscle at periphery of tumor.) × 10.

FIG. 42. Stages in development of adamantoblastomas. Mouse 51. Solid epidermoid adamantoblastoma. (A) Area of previous excision for biopsy. (B) Invasion into masseter muscles. (C) Oral ulceration with epithelial downgrowths. (D) Proliferation from enamel organ of incisor tooth. × 10.

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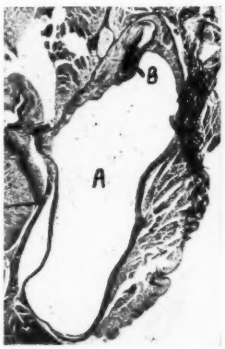

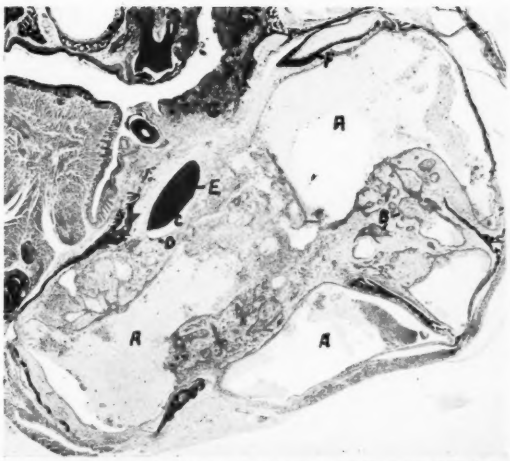

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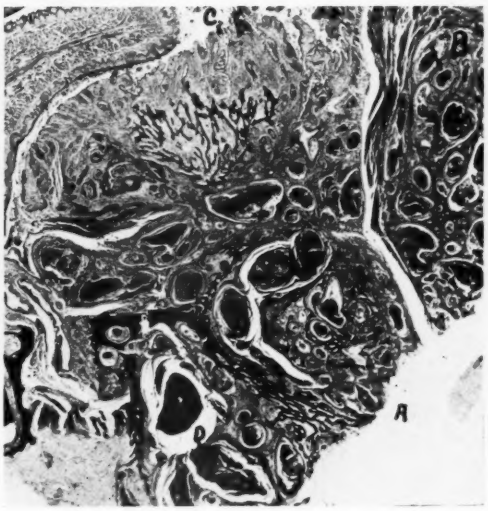

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Adamantoblastomas in the Slye Stock of Mice

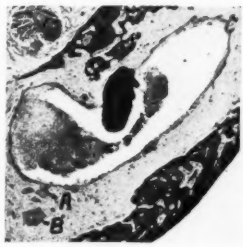
PLATE 16

FIG. 43. Stages in development of adamantoblastomas. Mouse 65. Multicystic epidermoid adamantoblastoma. × 10.

FIG. 44. Mural attachments to cystic linings. Mouse 9. (A) Mural proliferations from cystic lining. (B) Epithelial masses in stroma which have become severed from the cystic lining and which transform this tumor from a purely cystic to a cystic-solid growth. × 11.

FIG. 45. High-power photomicrograph of area in Figure 44. (A) Cystic lining. (B) Mural epithelial masses attached to the cystic lining. (C) Similar masses without demonstrable attachment. × 385.

FIG. 46. Sarco-adamantoblastoma. Mouse 21. (A) Sarcomatous portion of mixed tumor with giant cells at B. (C) Epithelial portion of mixed tumor. (D) Cystic space with inflammatory exudate. × 155.



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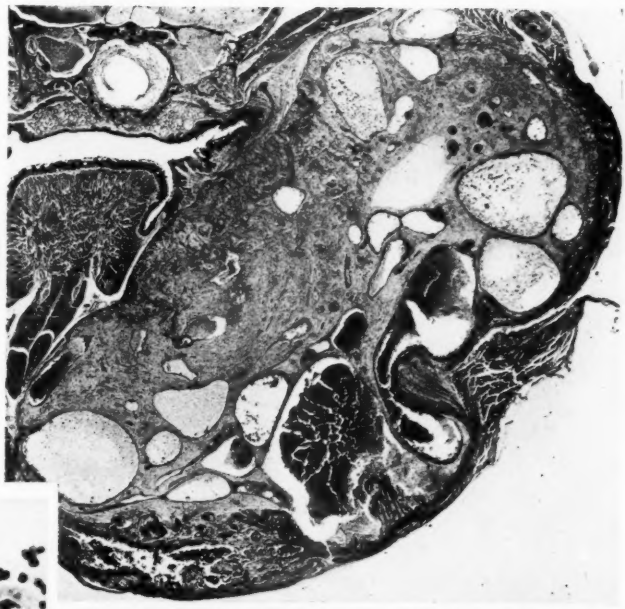

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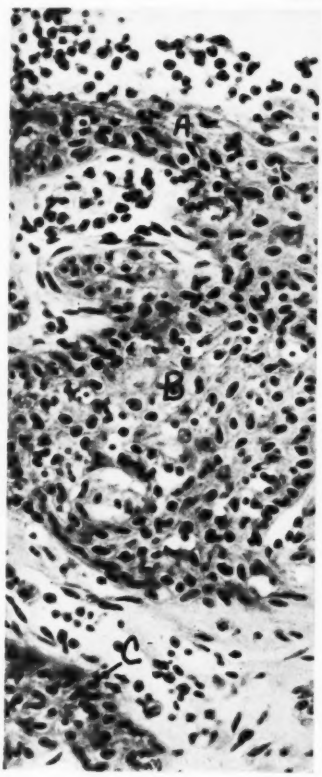

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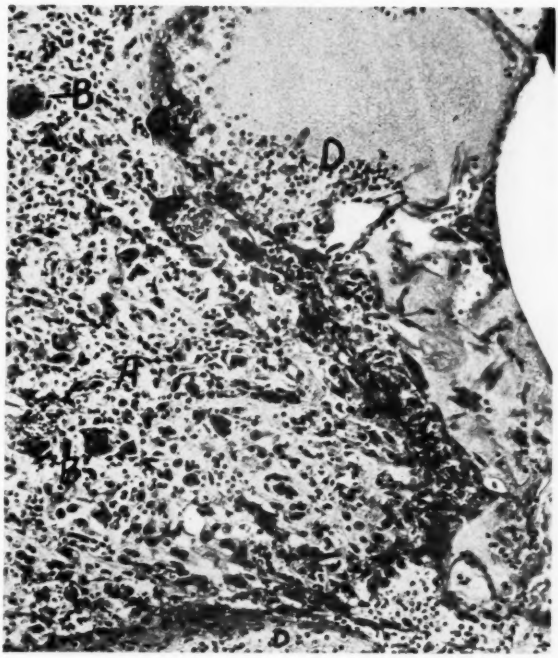

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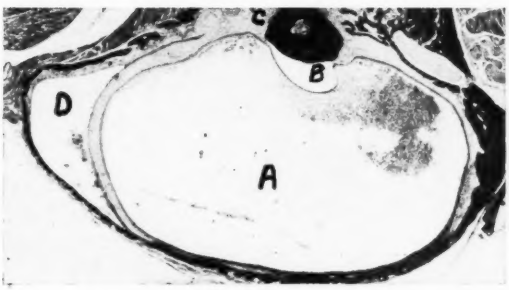
Adamantoblastomas in the Slye Stock of Mice

PLATE 17

FIG. 47. Odontoma. Mouse 11. Bizarre pattern of an odontoma formed by the irregular laying down of neoplastic tooth elements. (A) Enamel space. (B) Dentin. (C) Pulp tissue. There is little similarity to a normal tooth structure although almost all tooth elements, both epidermal and mesodermal, are involved in the tumor. × 13.

FIG. 48. Odontoma. High power of portion of Figure 47. (A) Enamel space with organic matrix. (B) Dentin. (C) Pre-dentin. (D) Odontoblasts. (E) Pulp. (F) Ameloblasts. (G) Enamel organ. Note the wild, bizarre arrangement of enamel, dentin and pulp structures with no regular tooth pattern discernible. The odontoma represents a tumor of *both* enamel and dentin, *i.e.*, of both epithelium and connective tissue. × 145.

FIG. 49. Monocystic adamantoblastoma which is similar to what is known as a dentigerous cyst in man. Longitudinal section. Mouse 64. (A) Cystic tumor surrounding the enamel space (B) of incisor tooth. (C) Peridental membrane uninvolved. (D) Auxiliary cyst which appears as an individual structure but is actually a lobule of the large cyst. × 11.



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Adamantoblastomas in the Slye Stock of Mice

EXPERIMENTAL THROMBOTIC BACTERIAL (STREPTOCOCCUS VIRIDANS) ENDOCARDITIS

I. Its Production and Incidence in the Rabbit *

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The primary objective of these experiments was to help resolve the problem of therapeusis in subacute bacterial endocarditis. The more rational and innocuous approach of animal experimentation appealed to us rather than direct evaluation of new expedients in human patients afflicted with the disease. It is true that countless therapeutic measures have been advanced for this disease, which alone attests to the futility of most of them.

For our purpose it is essential that the disease be reproduced consistently in a convenient laboratory animal. This initial report deals with methods and procedures adopted to achieve the greatest possible incidence of the experimental disease in rabbits.

Experimental endocarditis produced by bacteria other than *Streptococcus viridans* isolated from human cases has been reported many times.[1-3] There are also numerous reports of experimental endocarditis produced by traumatizing the endocardium or by the injection of particulate matter in addition to bacterial inoculations.[4-7]

Dreschfeld,[8] in 1887, was the first investigator to reproduce the disease without first resorting to mechanical injury of the cardiac valves. Horder [9] and Rosenow [10] also produced endocardial vegetations by simply injecting intravenously cultures of *Str. viridans*. The most recent work in this direction is the splendid accomplishment of MacNeal, Spence and Wasseen,[11] who demonstrated that it is possible to transmit endocarditis lenta of man to the rabbit by repeated intravenous injections of large amounts of pure cultures in serum-broth.

The technic followed was an adaptation of the pattern suggested by MacNeal and his co-workers.[11] Strains of *Str. viridans* were obtained by blood culture from clinically typical cases of subacute bacterial endocarditis. The organisms were subplanted in meat infusion broth which was occasionally enriched with rabbit plasma. One to 6 cc. of a fresh 24-hour culture was injected daily into an ear vein of a rabbit for 6 days. After an interval of 48 hours, a blood culture was taken and the sequence then repeated. This program was continued for variable periods until repeated positive blood cultures, progressive loss in weight and the development of cardiac murmurs heralded the presence

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of endocardial vegetations. Some animals died spontaneously, others were sacrificed. In some series attempts were made either to prevent or to cure the experimental disease. These results will be reported in a later communication. Necropsies were performed as soon after death as possible. Where vegetations were found, the organisms could be demonstrated histologically with ease, as well as in cultures. Mitral lesions predominated, although aortic, tricuspid and multiostial lesions were also encountered. Gross renal and splenic infarctions were observed as were the typical microscopic, embolic, renal lesions of Löhlein.[12]

The initial experiments were begun in August, 1940. For this series 31 rabbits and five strains of *Str. viridans* were employed. For these probatory experiments the organisms were grown in plain meat infu-

TABLE I

Strain	No. rabbits	No. positive	Per cent positive
M	1	1	100.0
R	6	3	50.0
S	7	1	14.0
H	5	0	0.0
A	2	1	50.0
M and R	10	5	50.0
Total	31	11	35.5

TABLE II

Strain	No. rabbits	No. positive	Per cent positive
S	7	3	42.8
H	8	8	100.0
B	6	4	66.6
Total	21	15	71.4

sion broth and only 1 to 2 cc. of the fresh 24-hour culture was injected. The results of this early experiment are shown in Table I.

For the purpose of this work the results in this series were too desultory and unpredictable. It was felt that the percentage of animals with endocarditis could be improved by stepping-up the virulence of our strains. With this in mind the H and S strains, which incidentally had given the poorer results, were passed through mice and cultured on broth enriched with rabbit plasma. In addition, the daily dose of inoculum was increased to 4 cc. Finally, a recently isolated and, presumably, more virulent strain was singled out and employed. The more helpful response is noted in the experiments summarized in Table II.

The inferences to be drawn from these experiments are that different strains of *Str. viridans* vary in their capacity to produce endocardial lesions in the rabbit, and, further, that this potentiality can be enhanced by passage of weak strains through mice, or by growth in enriched mediums, or both.

This thesis was confirmed in the next series of experiments which were begun in May, 1941. Streptococcal strains H and B, which were permitted to deteriorate by transplanting in plain meat infusion broth, were employed, as were two newly recovered strains of *Str. viridans*, F and G. The results are summarized in Table III.

Thus, endocarditis was produced in only 4 of 15 rabbits with the use of the devitalized strains H and B. On the other hand, fresh, relatively virulent strains F and G produced lesions in 8 of 11 animals.

TABLE III

Strain	No. rabbits	No. positive	Per cent positive
H	7	3	42.8
B	8	1	12.5
F	5	5	100.0
G	6	3	50.0

CONCLUSIONS

1. The rabbit is a suitable and convenient laboratory animal for the experimental production of subacute bacterial endocarditis.
2. A simple technic is outlined for the production of the disease in rabbits.
3. Different strains of *Str. viridans* vary in their ability to produce the experimental disease in the rabbit.
4. The virulence of weak strains of *Str. viridans* can be enhanced by passage through mice and by culturing on enriched mediums.
5. Subacute bacterial endocarditis was produced in from 50 to 100 per cent of rabbits in adequately controlled experiments.

We are indebted to Mr. Mortimer Russell for his technical assistance in this study.

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DESCRIPTION OF PLATE

PLATE 18

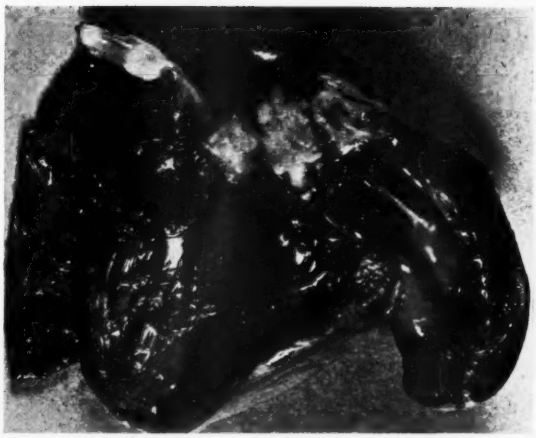
FIG. 1. Rabbit 903. Thrombotic endocarditis of tricuspid valve. This was combined with a mitral lesion. × 1.4.

FIG. 2. Rabbit 903. Thrombotic endocarditis of mitral valve. (See Fig. 1.) × 1.2.

FIG. 3. Rabbit 16. Thrombotic endocarditis of aortic valve. × 1.4.

FIG. 4. Rabbit 631. Infarct in kidney. Heart revealed aortic endocarditis. × 1.6.

FIG. 5. Rabbit 13. Experimental thrombotic endocarditis involving the aortic valve, showing complete destruction of cusps and huge vegetation harboring clumps of streptococci. × 20.



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PROGRESSIVE EXPERIMENTAL ENDOCARDITIS LENTA *

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In a previous paper [1] we considered some of the very early changes observed in the hearts of rabbits after they had received repeated large intravenous inoculations of *Streptococcus viridans*. Such animals have shown evidence of a widespread infection of the endothelial cells of the endocardium and a tendency for the infection to progress to more serious destruction in certain locations, especially on the auricular faces of the mitral and tricuspid leaflets, on the ventricular faces of the aortic cusps, on the intima of the ascending aorta and in the myocardial capillaries. When the experimental animals survive for longer periods, *i.e.*, 1 to 10 weeks after the initial inoculation, there is found abundant evidence that the original widespread infection has been overcome by the local defense mechanisms in many places, in some locations with apparently complete restitution and at other sites only at the expense of more or less serious structural alteration, while in still other localities the infectious process persists and eventually leads to local alterations and to distant complications, such that the life of the animal can no longer continue. During this progressive stage of the disease there are thus presented apparently conflicting phenomena of healing and of destruction. In the present paper we purpose to invite attention especially to the destructive tissue alterations on the valve leaflets and their relationship to the behavior of the bacterial invaders, deferring for the most part the consideration of repair and healing.

In a great majority of our experimental animals, now numbering many hundreds, the disease has progressed to death. The most conspicuous gross lesions are the vegetations on the mitral, aortic and tricuspid valve leaflets, on the mural endocardium, on the aortic intima and rarely on the pulmonary semilunar cusps. The vegetations in the rabbit are relatively larger than those ordinarily seen in the human heart. Sometimes the animals survive until the stenosing mitral mass of morbid material seems to obstruct almost completely the passage from auricle to ventricle. The finer structure of these vegetations is

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variable but essentially of the same nature as that seen in the human lesions.[2] In addition to the valvular vegetations, there are found also septic lesions of the kidneys and lungs, similar to those in man, enlargement of the spleen, usually without infarction, and interesting alterations in blood vessels and in the myocardium, some of which are still only imperfectly elucidated. There is considerable variation in different animals in respect to character, location and rate of development of the lesions. Nevertheless, some changes are seen especially in the early days or weeks of the infection and others are found chiefly in animals ill for a month or more.

Rabbit 4 of series 10 weighed 3600 gm. on May 10, 1939. It was inoculated intravenously with pooled culture strains of *Str. viridans* isolated from four patients: Pl, Fi, Ha and Ho. The bacteria were grown in rabbit-serum broth, sedimented by centrifugation, resuspended in saline solution, pooled and injected into the ear vein. The bacterial growth of 20 cc. of the serum broth was represented by 2 cc. of the final suspension. Rabbit 4 received 0.5 cc. of such pooled suspension daily on May 11, 12 and 13, a total of 1.5 cc. It died at 11:00 a. m. on May 15, only 4 days after the initial inoculation. At necropsy there was found a small vegetation on the tricuspid valve and a slightly larger one on the mitral valve.

A section through the mitral valve is represented by the photomicrograph, Figure 1. The original substance of the leaflet is swollen and distorted and on its auricular face there is a thick deposit of fibrin, in part so friable as to be fragmented in the section. Wandering cells are abundant in the substance of the leaflet just beneath the fibrin and collections of wandering cells are present also in some parts of the fibrin deposit itself. For the most part, however, the latter is relatively or entirely free from nucleated cells. Colonies of streptococci are abundant and irregularly distributed throughout the mass of fibrin. Some bacterial colonies are found near the substance of the leaflet proper, but not in it. The most superficial layer of fibrin also appears to be free from bacterial colonies. The colored drawings, Figures 4a, 4b and 4c, represent a narrow strip in this same section extending through the substance of the leaflet from the ventricular endothelial surface (a) to the deepest portion of the fibrin deposit on the auricular aspect, in which the deep group of bacterial colonies can be recognized in the photomicrograph (Fig. 1). The top colored strip (a) shows the ventricular surface of the leaflet, where the endothelial cells are swollen, somewhat basophilic and apparently increased in number. Beneath them, the connective tissue is edematous and has in it polymorpho-

nuclear leukocytes, some eosinophils and lymphocytes. The second colored strip (b) carries on to the right of strip a, showing the mid-portion of the leaflet proper. Here the edema is greater and the cellular infiltration somewhat less. Toward the right end of the strip there are more fragmenting leukocytes. The third strip (c) carries on to the right of strip b. Here the wandering cells have become more abundant and the infiltration with polymorphonuclear leukocytes extends almost to the necrotic auricular surface of the leaflet, where it is intimately adherent to the fibrinous vegetation, containing colonies of streptococci. There is an appreciable interval of cell-free fibrin separating the bacterial colonies from the stainable nucleated tissue cells and leukocytes. Apparently the large bacterial colonies, during the rapidly progressive stage of their growth in fibrin, are able to keep the living body cells at a distance. On the other hand the endothelial cells of the endocardium at this stage seem to have become altered so that they either fail to phagocytize the isolated cocci which are circulating in the blood or, more probably, have acquired the ability to digest and dissolve the streptococci quickly after they have been phagocytosed so that the bacteria are not found in them at this stage of the disease.

Rabbit 621, series 18, weighed 2580 gm. on November 21, 1939. It was injected intravenously with immune serum from November 21 to December 1 inclusive, 10 cc. daily except November 26, to a total of 100 cc. This serum was derived from rabbits immunized against *Str. viridans* and it possessed a high agglutination titer. Subsequent to the start of the serum injections, rabbit 621 was inoculated intravenously with a suspension of bacterial culture, *Str. viridans*, strain Cr., which had been grown 18 hours in broth, centrifugalized and suspended in physiological saline solution, the bacterial growth of 20 cc. broth culture being represented by 2 cc. of the final suspension in saline solution. The rabbit received 2 cc. of such suspension daily on November 23, 24, 25, 27, 29 and 30 and on December 1, a total of 14 cc. It died on December 2, 1939, 9 days after the initial bacterial inoculation. At necropsy there was found an irregular rough vegetation on the tricuspid valve and a rough deposit, 9 by 6 by 4 mm. in diameter, on the right mural endocardium near the right border of the heart. On the left side the mitral orifice was occupied by a vegetation measuring 8 by 6 by 6 mm. The aortic and pulmonary valves appeared negative. A gross photograph of the opened heart is shown in Figure 2.

A section passing through the mitral valve is represented by photomicrograph in Figure 3. One can recognize the deformed and thickened structure of the valve leaflet and the large, irregular and somewhat

fragmented mass of fibrin deposited on its auricular face, but also extending onto the ventricular surface of the valve and onto the ventricular wall at an area of contact. Many nucleated cells are present in this vegetation and also many colonies of streptococci. The latter appear in the photomicrograph as black spots of irregular size and outline, especially numerous near the ventricular surface of the mass. The colored drawing, Figure 5, shows a small bit of the ventricular wall where the friable vegetation is attached. Streptococci can be recognized only in the dense colonies and one searches in vain for individual cocci in the endocardial exudate, in the altered endothelium and also in the subjacent muscle which has been infiltrated by exudate.

Rabbit 822, series 26, weighed 2620 gm. on October 9, 1939. It was given intravenous injections of *Str. viridans*, strain Ep., 2 cc. of the usual type suspension daily on October 9, 10, 11, 12, 14, 15 and 21, to a total of 14 cc. Beginning on October 22, sodium sulfathiazole was given intravenously, 50 mg. per day on October 22, 23, 24, 25, 26, 28, 29, 30 and 31 and on November 1. The body weight was 2370 gm. on October 14, 2070 gm. on October 21 and 1900 gm. on October 28. The blood culture taken on October 14 was negative, that of October 21 positive and, again, that of October 28 positive. Auscultation on October 30 and again on November 1 detected abnormal heart sounds. The animal was found dead on the morning of November 2, 24 days after the initial bacterial inoculation. At necropsy there was abundant fluid in the peritoneal cavity; the heart was enlarged and the mitral ring was almost completely obstructed by massive vegetations.

A photomicrograph of a section through one of the mitral vegetations is shown in Figure 6 and the colored drawing, Figure 7, represents a small portion at the free surface of this same section. Apparently this is an example of a very malignant, actively progressive lesion on the valve leaflet. The bacterial colonies are seen extending to the free surface of the vegetation and evidently disintegrating here so as to free abundant streptococci into the circulating blood at every beat of the heart. The subjacent fibrin in this particular part of the vegetation is devoid of wandering cells and the bacterial colonies appear to be flourishing here without hindrance.

Rabbit 100, series 5, weighed 2090 gm. on February 10, 1939. It was given a series of intravenous injections of heat-killed bacterial vaccine made from strain Fi., *Str. viridans*, three times a week from February 10 to April 10 inclusive. The initial dose was ½ billion and the last five doses were each 10 billion bacterial cells. The animal lost weight to 1950 gm. on February 20 but then gained steadily to 2610

gm. on April 10. The total bacterial cells used for this preliminary immunization amounted to 120 billion. After an interval of 10 days the intravenous inoculations of living bacterial culture were begun on April 20, employing a suspension of the same strain of bacteria in saline solution, of which 2 cc. represented the growth in 10 cc. of serum-broth medium in 18 hours. This was given as follows: 2 cc. on April 20; 4 cc. on April 21; 5 cc. on April 22, 24 and 25; and 5 cc. on May 4, 5 and 8. The total amount of inoculum was 36 cc. The animal weighed 2640 gm. on April 24, 2415 gm. on May 1 and 2450 gm. on May 8. Blood cultures taken on April 24 and 27 were positive, those taken on April 29 and May 1 were negative and the final one taken on May 8 gave positive growth. The rabbit died on May 17. At necropsy there were large vegetations on the aortic ring.

A photograph of the vegetations is shown in Figure 8 and a section through the aortic vegetation is represented by the photomicrograph in Figure 9. There is a large mass of fibrin containing abundant bacterial colonies situated on the ventricular surface of the leaflet and there is similar morbid material attached to the adjacent ventricular endocardium. The colored drawing, Figure 10, depicts a small area of the section at the free border. At this place the deeper portion consists of cell-free fibrin in which there are abundant flourishing colonies of streptococci and many vacant clefts. Over this deepest layer there is a more compact layer of fibrin containing fragmented and deformed wandering cells and apparently free from bacteria. This is overlaid by a third stratum, of recent origin, in which abundant, well preserved erythrocytes and leukocytes can be recognized in the clot. Apparently this layer had been deposited during the last hours of life. On the other hand, the layer containing the deformed leukocytes would seem to have been deposited earlier and to indicate an attempt to wall in the infected mass by fibrin into which the bacteria could not readily penetrate. The appearance suggests, therefore, a somewhat greater tendency to resist the infectious agent than that evident in rabbit 822.

Rabbit 655, series 16, weighed 2420 gm. on October 9, 1939. It was inoculated intravenously with culture strain Cr., *Str. viridans*, the daily dose representing the centrifugalized growth of 18 hours in 10 cc. serum broth, separated and suspended in 2 cc. of physiological saline solution, or multiples of this amount. At the same time rabbit serum possessing a high agglutinin titer against the streptococcus was injected intravenously. The doses are indicated in Table I. The animal died on November 7. At necropsy there were found small rounded vegetations on the mitral leaflets and a large vegetation on the aortic valve.

The gross appearance is shown in Figure 11. A photomicrograph of a section passing through the aortic valve and the ostium of a coronary artery is shown in Figure 12. The aortic leaflet is partly covered on both surfaces by a mass of relatively cell-free fibrin in which there are abundant crowded colonies of streptococci growing out to the free surface of the vegetation. On the adjacent wall of the aorta there is a large intimal plaque made up for the most part of bacterial colonies. Beneath this the media of the aorta is swollen by edema fluid and is superficially invaded by the bacterial growth. A small portion is represented in detail in the colored drawing, Figure 13. Here it seems that the bacterial colonies have been able to digest the collagen and elastin of the aortic wall so that they penetrate into it and, at the same time, to produce necrotizing substances which cause disappearance of neighboring cell nuclei and edema of the more distant tissue elements.

TABLE I

Protocol of Inoculation and Treatment of Rabbit 655

Date period 1939	Weight	Blood culture	Daily inoculum	Daily serum dose
	gm.		*cc.*	*cc.*
Oct. 9	2420			
Oct. 10			2	5
Oct. 11 to 14			4	5
Oct. 16 to 21	2500	Negative	4	5
Oct. 23	2370	Negative	4	5
Oct. 24 to 28			6	5
Oct. 30 to Nov. 4	2250	Negative	6	5
Nov. 6	2120	Positive	None	None
Total amounts			112	115

From the study of the progressive types of vegetations on the cardiac valves of the rabbits, only a few of which can be illustrated and described, it is possible to form a fairly definite idea of the general course of events at these sites. The initial bacterial invasion of the blood stream is followed by phagocytosis of the streptococci, a process in which the endothelial cells lining the blood vascular channels play a prominent rôle. The biological factors which favor or inhibit the phenomenon of phagocytosis at a particular site or at a particular time are only imperfectly understood. The phenomenon is by no means limited to the heart, for it seems that the intimal lining of vascular channels in the liver, spleen and lungs is even more actively concerned in this phagocytosis. In the heart itself the blood vessels of the myocardium are seriously involved in the removal of the streptococci. However, our immediate present attention is directed to the participation of the endocardium and especially to that of the valve leaflets.

Most of the streptococci appear to be killed and digested by the phagocytic endothelial cells which become somewhat swollen and lose their normal, relatively thin, flat form to assume a more rounded contour. They are in part destroyed also, but in some places they are stimulated to multiply by mitotic cell division. If complete destruction of the microbes is accomplished for each succeeding invasion of the blood, then the animal escapes development of bacterial vegetations. This has evidently taken place in a considerable number of the experimental rabbits, even after repeated inoculations. In them the restitution of the endothelium may be so complete that evidence of injury may not be recognized. On the other hand, the matter turns out otherwise when the active virulence of the bacteria is relatively great and the resistance of the animal naturally weak or lowered by other disease such as severe coccidiosis. In such, the streptococcal infection may overwhelm the host in the early bacteremic stage before local gross vegetations arise. Between these two extremes there is produced a less rapidly fatal infection in which the vegetations characteristic of bacterial endocarditis are conspicuous features. The mitral valve is the most common location and the favorite area is along the line of contact where the leaflets are pressed against each other during ventricular systole, that is, on the auricular faces near the free margins. Corresponding relatively vulnerable areas may be recognized on other valves.

At such a susceptible site the initial infection of the endothelial cells is followed by their swelling and the accumulation of edema fluid in the stroma beneath them. The injury to the endothelial cells may cause their separation so as to expose the subjacent stroma or it may serve merely to release sufficient thromboplastic elements to bring about a deposit of platelets and fibrin on the surface without loss of the endothelial coat. In either event the fibrin deposit, once started, tends to increase. In its meshes are included streptococci which find in it a favorable medium for the production of large bacterial colonies. One may well agree with the usual statement that *Str. viridans* is an organism of low virulence, but nevertheless when these large colonies are growing rapidly in the fibrin they produce substances which are ordinarily able to keep at a distance any living body cells and these bacterial products bring about a remarkable hydrophilic change in the neighboring connective tissues, manifest by edematous swelling and fragmentation of their structure in addition to the accumulation of various wandering cells and the proliferation of the fixed elements. The valve proper thus becomes greatly thickened. The fibrinous deposit on its surface continues to add more and more fibrin from the blood stream at its free

surface while the streptococci multiply in its substance. Red and white cells may be included, coming from the blood stream. The free surface of such a progressive vegetation is jagged, irregular and easily broken so that fragments of the fibrin and bacteria from the actively growing colonies near to and on the free surface may be set free into the general circulation.

The underlying stroma of the original leaflet may undergo partial necrosis or it may respond by proliferation of its elements. In most lesions of longer standing both phenomena are represented by necrosis at one place and fibroblastic proliferation at another. Remarkable deformity and intermingling of infected fibrin, necrotic and suppurating stroma, proliferating fibrous tissue and proliferating endothelium may thus be produced, resulting in a confused and confusing arrangement similar to that often seen in the human lesions of longer duration. The inflammatory changes in the living stroma may be associated with the formation of blood spaces lined by endothelium or of genuine blood capillaries, elements which, in the human lesions, have supplied a topic for controversy.

In some rabbits, because of greater resistance of the animal or relatively lesser virulence of the infection, the necrotizing effect is diminished and the reparative exudation and proliferation relatively enhanced. Sometimes the new layer of fibrin is relatively free from bacteria for a space and then again heavily infected on or near the surface so that the vegetation presents a succession of layers. In such a vegetation the deeper colonies are often made up of minute, poorly staining cocci, apparently relatively suppressed. Over them the layer of bacteria-free fibrin indicates restraint of bacterial growth in this layer. Then on top of this there may be a fresh deposit of fibrin rich in flourishing bacterial colonies in which the individual cocci are relatively large and stain very well. Such lamination evidently indicates a period of relative resistance followed by a recidive of infectious activity. The more or less successful opposition to the microbes seems to depend especially upon unseen soluble elements in the blood which tend to hamper rapid bacterial growth, and the most conspicuous early effect of this is manifested by the deposit of a bacteria-free coat of fibrin on the free surface of the vegetation so as to bury the bacterial colonies and keep them from the circulating blood. Over this layer of bacteria-free fibrin the endothelial cells of the adjacent endocardium may extend to form a smooth protective coat, and into the fibrin may grow proliferating fibroblasts, much as such cells grow into the fibrin of a tissue culture *in vitro*. These changes suggest the nature of a possible heal-

ing process and will require renewed consideration when we discuss the arrested and healing stages of the experimental disease. They are, however, seen here and there in some animals in which the disease progresses actively to a fatal termination. Even when the infecting bacterium seems to have been brought under restraint, the structural damage already inflicted may result in lethal dysfunction.

The aortic valve, which is the second most frequent site of large vegetations, shows changes essentially similar to those seen on the mitral leaflets. Here the vegetation appears to start by preference on the ventricular surface of the leaflet, again at the line subjected to pressure against the endothelium of a fellow cusp. The progress of the aortic vegetation is like that of the mitral but there is here apparently a greater tendency for bacterial growth to extend onto the adjacent aortic intima and into the coronary arteries and also a greater tendency for gross fragments of vegetations to escape into the aortic blood stream.

On the tricuspid leaflets the vegetations are usually smaller, often multiple and separate and they tend to be smoother and less friable than those of the left heart. Organizing fibrosis and covering by smooth endothelium are more frequently seen here, in our experience. Apparently the less violent pressure changes favor the healing tendency but one cannot be certain whether the concentration of carbon dioxide and deficiency of oxygen play any part. Healing tricuspid vegetations may be found in the same heart along with violently progressive lesions on the mitral and aortic leaflets.

Vegetations on the tendinous cords and papillary muscles occur more frequently in the right heart but they are more destructive when they are found in the left ventricle, where the chordae tendinae are severed not infrequently. In the right ventricle, on the other hand, one often finds evidence of healing in the vegetations on the tendinous cords.

Changes in the mural endocardium are of special interest. The initial diffuse infection of this endothelial layer is rarely recognized and in general it seems to be of relatively brief duration in the rabbit. Apparently this diffuse infection is rapidly overcome by the intracellular digestion of the phagocytosed streptococci, without recognizable permanent morphological change in the endothelium. However, its functional relation to the bacteria appears to have been altered as a result of this experience so that subsequently the bacteria either are not phagocytosed or if phagocytosed are rapidly annihilated. At any rate one searches in vain for microscopical evidence of general bacterial infection of the mural endothelium of animals dying during the later weeks of the

disease. In some locations the early destruction of the phagocytosed bacteria is associated with endothelial and subendothelial injury, probably because the disintegrating bacterial cells liberate too much toxic material and some of the microbes escape destruction and are able to multiply in the injured endothelial cells and in the fibrin deposited over them. The structural change may include edematous swelling and fragmentation of subjacent connective tissue and heart muscle fibers, along with accumulation of wandering cells even though, at the time, streptococci have disappeared or have become so few that they escape microscopical detection. In other instances, more serious local alteration permits luxuriant proliferation of the bacteria in the superficial layers of necrotic tissue and in the local fibrin deposit, giving rise to a mural vegetation by a mechanism much the same as that operating on the valve leaflets. The mural lesions are subjected to less physical strain and trauma and they show greater tendency toward arrest and healing.

The changes in the coronary arteries and veins, and in the myocardial capillaries and in the myocardium proper seem worthy of separate discussion. Alterations in the aorta and in other blood vessels as well as the changes observed in the kidney, spleen, liver and lung can receive only passing mention. Their study in comparison with lesions observed in man appears to offer important possibilities.

Summary

1. By repeated intravenous injection into rabbits of large doses of living streptococci derived from the blood of patients afflicted with endocarditis lenta it has been possible to cause, in a large proportion of these animals, a fatal disease resembling in its clinical course and anatomical alterations the disease of man.

2. The streptococci are phagocytosed by the endothelial cells in general and are evidently effectively disposed of by these cells, for the most part. They may, however, succeed in destroying the endothelial cells in certain places, especially on the valve leaflets where these cells are subjected to contact pressure when the valve is closed.

3. Serious local injury of endothelium results in a local deposit of fibrin and in this the streptococci tend to flourish to induce further toxic injury to the underlying structure of the leaflet and more deposition of fibrin from the blood.

4. The fluid blood may exercise a restraining influence and may prevent the bacterial colonies from growing in the outermost layers of the vegetation. On the other hand, this restraint may fail and the streptococci then grow out in abundance to the free surface and escape into the circulating blood.

5. Evidence of a period of relative restraint has been observed in some of the rabbits, a period succeeded by further rapid progress of the disease process on the valve.

6. The changes which tend toward healing of the valve lesions, the changes in blood vessels and in the myocardium and the lesions in other organs and tissues require further study.

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2. MacNeal, W. J., Spence, M. J., and Wasseen, M. Experimental production of endocarditis lenta. *Am. J. Path.*, 1939, **15**, 695–705.
3. Brown, J. H., and Brenn, L. A method for the differential staining of Gram-positive and Gram-negative bacteria in tissue sections. *Bull. Johns Hopkins Hosp.*, 1931, **48**, 69–73.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 19

FIG. 1. Photomicrograph of section through mitral valve of rabbit 4, series 10, which died 4 days after initial inoculation with *Streptococcus viridans*. Hematoxylin and eosin stain. The swollen and distorted valve leaflet is covered on its auricular surface by a mass of friable fibrin in which there are numerous colonies of streptococci and some large collections of wandering cells. The narrow strip through the valve leaflet is shown in detail in Figures 4a, 4b and 4c.

FIG. 2. Photograph of the opened heart of rabbit 621, series 18, which died 9 days after initial inoculation with *Streptococcus viridans*. In the right ventricle there are several vegetations which are rather smooth and white and in part located on the ventricular wall. On the left side the mitral ring is nearly filled with rough, partly excavated and discolored vegetations.

FIG. 3. Photomicrograph of section through mitral vegetation of rabbit 621, stained by the method of Brown and Brenn.[a] Both auricular and ventricular faces of the valve leaflet are covered by friable vegetations which contain abundant streptococci, especially in the portion on the ventricular surface. The adjacent mural endocardium of the ventricle has become infected, apparently by contact with the vegetation on the leaflet. A small area of this invaded mural endocardium is shown in more detail in Figure 5.

1

2

3

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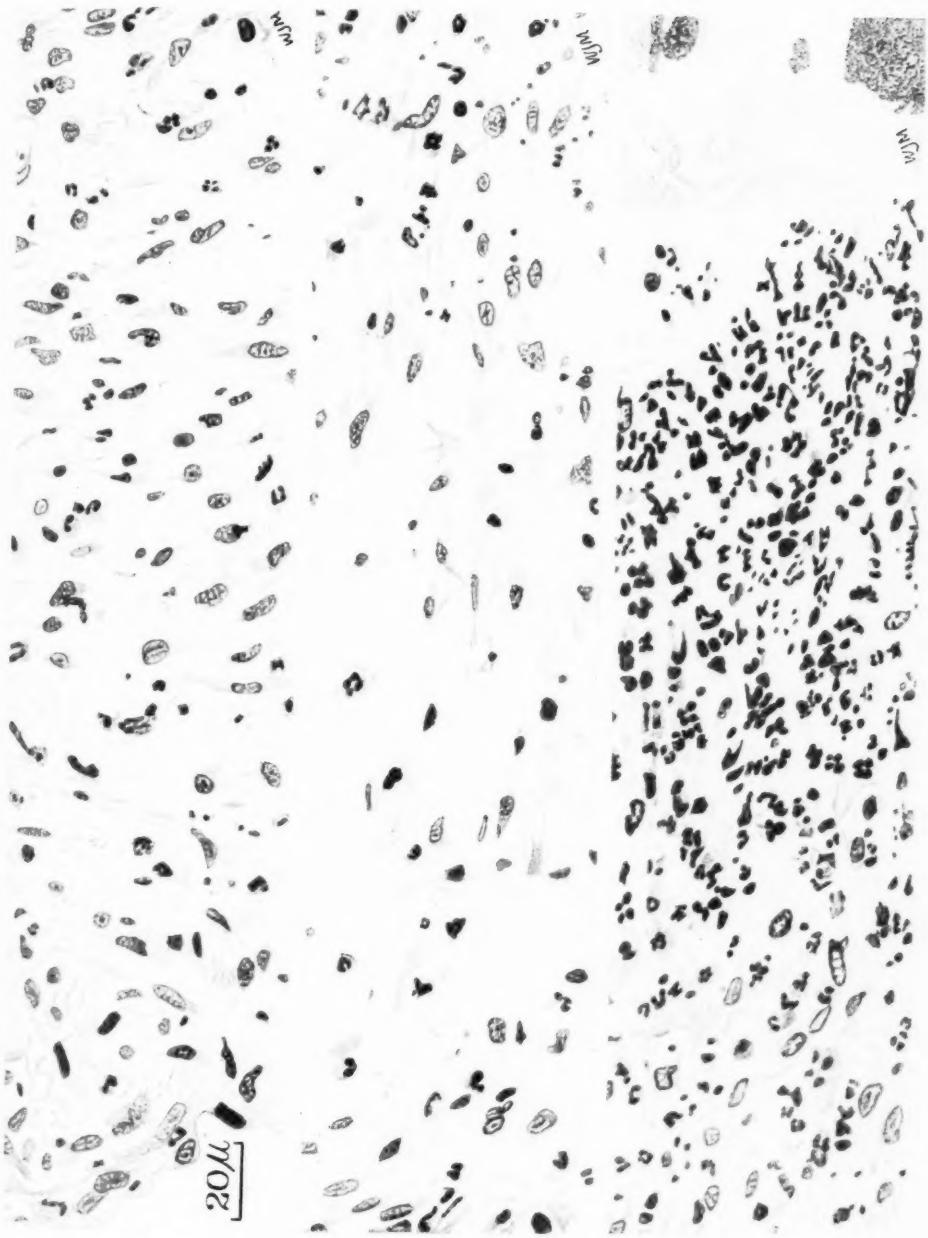
PLATE 20

FIG. 4a. The ventricular third of a narrow strip through the valve leaflet proper. At the left end are swollen endothelial cells of the ventricular surface of the leaflet.

FIG. 4b. The succeeding middle portion of the same narrow strip. In this part there is more edema, and fewer wandering cells are present.

FIG. 4c. The succeeding portion of the same narrow strip extending through the leaflet proper to include a small bit of the deepest part of the fibrinous vegetation deposited on its auricular surface. Wandering cells become very abundant as one approaches the infected vegetation but they remain at a respectful distance from the colonies of streptococci in the fibrin. The endothelial cells of the auricular surface of the valve have been destroyed. The three drawings overlap slightly at their junction lines to permit orientation.



4A 4B 4C

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PLATE 21

FIG. 5. A small portion of the ventricular mural lesion at the location indicated on Figure 3. The muscle bundles are separated by edema and are, to some extent, infiltrated by wandering cells. The endothelial lining has been largely replaced by fibrinous deposit containing abundant, partly fragmented, wandering cells. The streptococci are recognized only in the rather large, compact colonies lying in the fibrinous deposit.

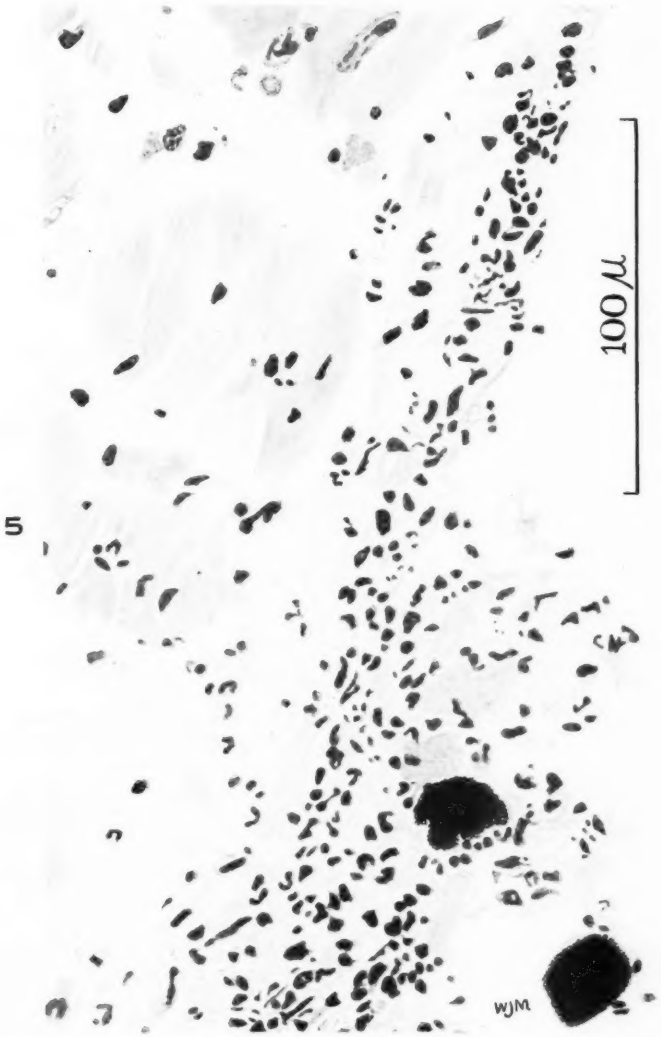

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PLATE 22

FIG. 6. Photomicrograph of section through a mitral vegetation of rabbit 822, series 26, which died 24 days after the initial inoculation with *Streptococcus viridans*. The swollen and deformed leaflet is largely covered with vacuolated fibrinous deposit, in which there are large collections of nucleated wandering cells, appearing as very dark areas in the illustration, and also very abundant colonies of streptococci, appearing less dark, irregularly distributed through the fibrin. A small area on the free ventricular surface of the vegetation is shown in more detail in Figure 7. Hematoxylin and eosin stain.

FIG. 7. A small superficial portion of the vegetation shown in Figure 6. Here the substance consists of fibrin and abundant colonies of streptococci without any recognizable nucleated cells. The bacterial colonies extend to the free surface so as to permit easy escape of the streptococci into the circulating blood. A small amount of blood is held in a shallow depression near the right side of the upper border of the figure.

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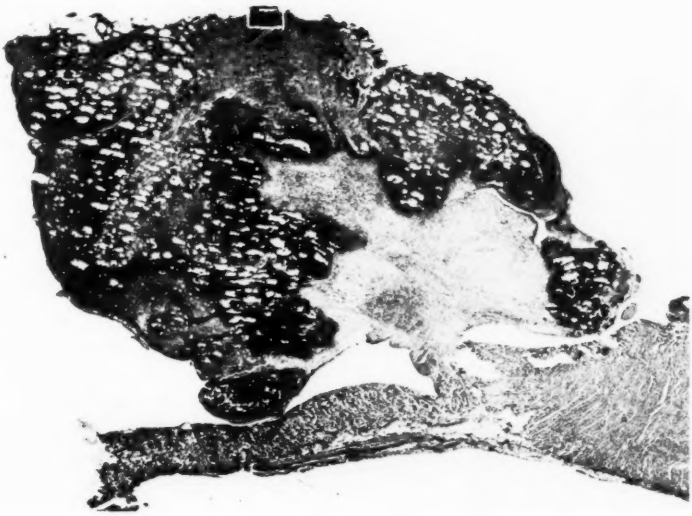

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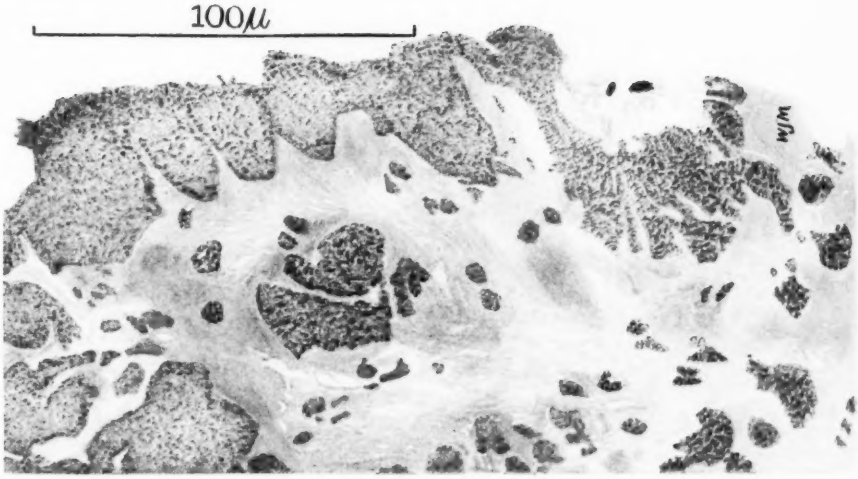

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PLATE 23

FIG. 8. Photograph of the opened heart of rabbit 100, series 5, which died 27 days after the initial inoculation with *Streptococcus viridans*. Here the mitral valve is not involved and the aortic ring is filled with rough, partly excavated vegetations.

FIG. 9. Photomicrograph of a torn and wrinkled section through the aortic valve of rabbit 100. The leaflet is hardly recognizable. In the fibrin there are abundant bacterial colonies, which are for the most part in the interior of the mass. Hematoxylin and eosin stain A small portion at the free surface is shown in more detail in Figure 10.

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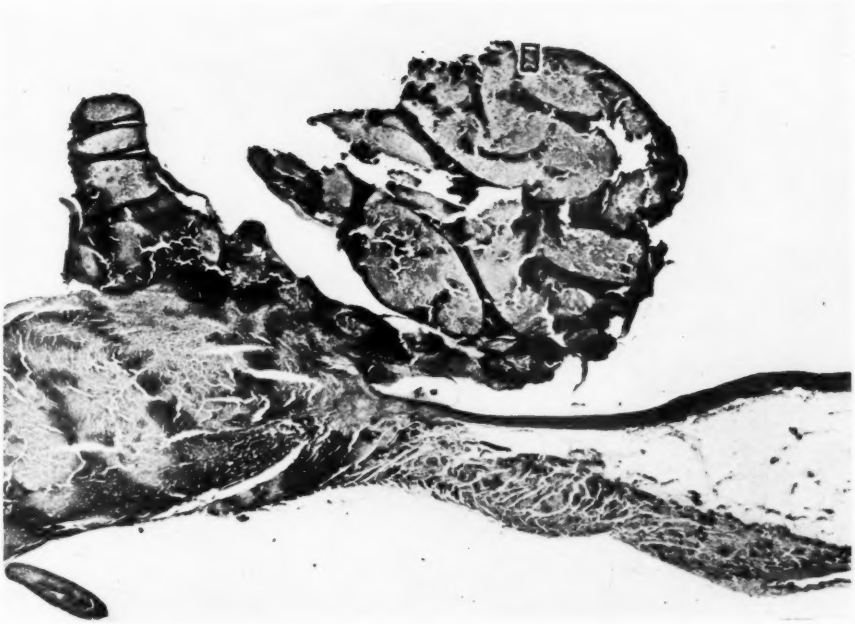

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PLATE 24

FIG. 10. A small superficial portion of the vegetation shown in Figure 9 In the deepest portion at the right the substance consists of cell-free fibrin and large bacterial colonies. Next to this there is a transition zone in which the bacterial colonies and nucleated wandering cells are closely associated, followed to the left by a zone of fibrin and fragmenting leukocytes without recognizable bacteria. Lying on this is more recent clot in which the blood corpuscles are well preserved.

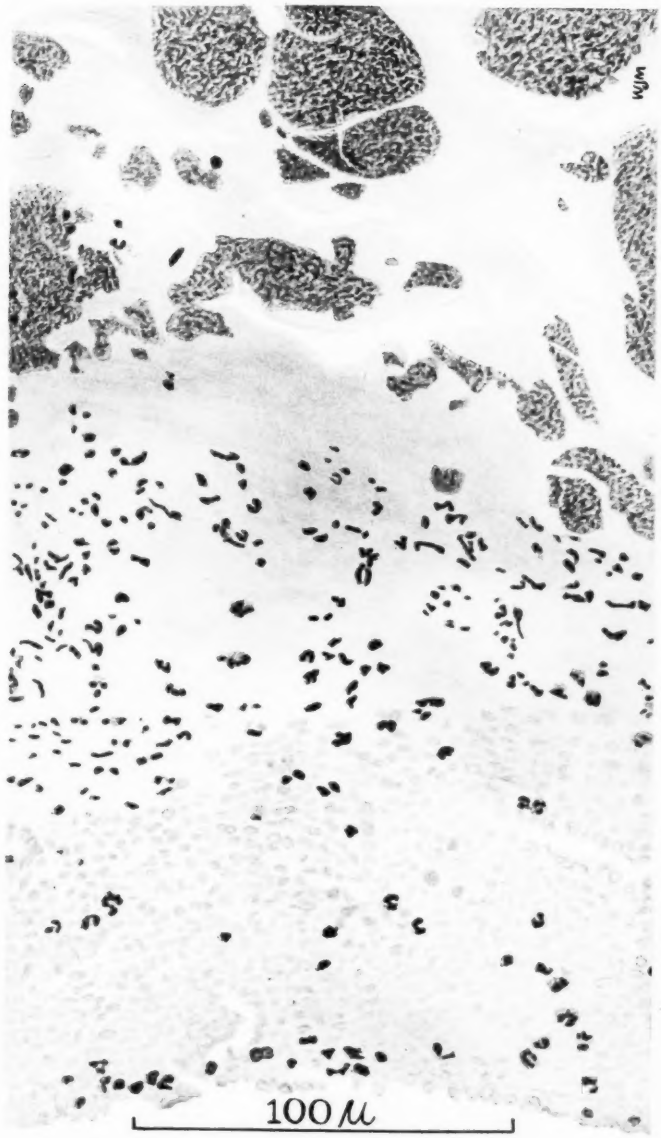

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PLATE 25

FIG. 11. Photograph of the opened heart of rabbit 655, series 16, which died 28 days after the initial inoculation with *Streptococcus viridans*. There are small, smooth, white vegetations on the mitral valve leaflets and the aortic ring is filled with large, partly excavated and discolored masses.

FIG. 12. Photomicrograph of a section through the aortic valve of rabbit 655, stained by the method of Brown and Brenn.[3] The leaflet is covered on both surfaces by the fibrinous vegetation with closely crowded colonies of streptococci presenting everywhere on the free surface. The bacterial colonies also form a thick layer on the adjacent aortic intima along which they approach the entrance to a coronary artery. The underlying media of the aorta is somewhat swollen by edema.

FIG. 13. Drawing of a small portion of the aortic wall in relation to the massive colonies of streptococci at the point indicated in Figure 12. Here the bacterial colonies appear to invade and actually to replace the superficial elastic lamellae of the aortic wall and to cause disappearance of the nuclei in their immediate vicinity. Farther from the bacterial colonies the elastic lamellae are fragmented by edema and invaded by wandering cells. However, a deep invasion by the bacteria has been prevented.

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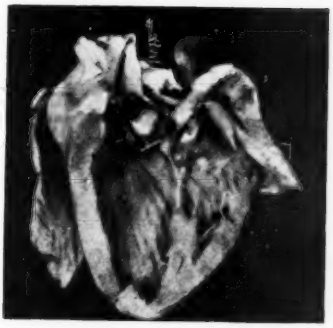

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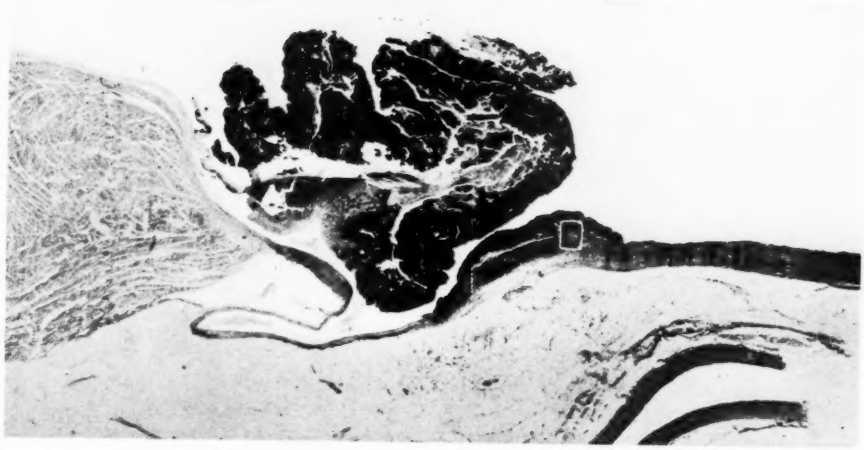

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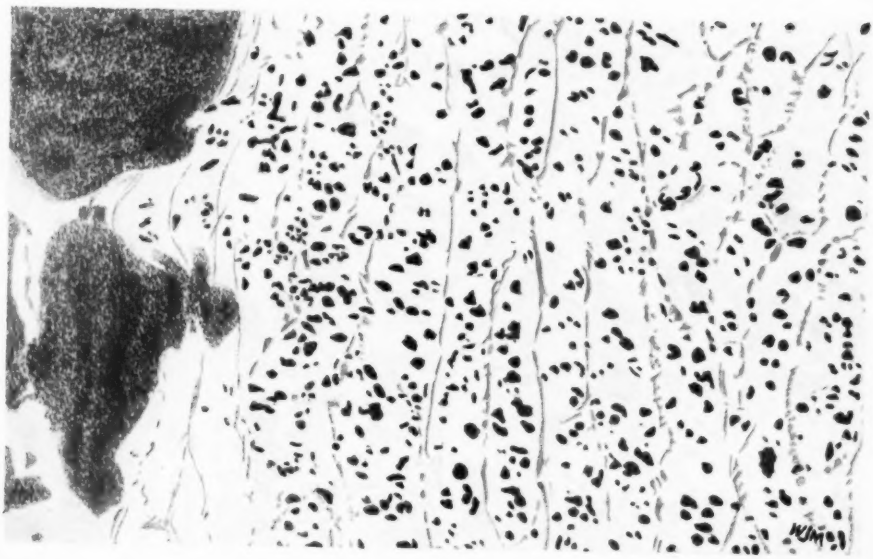

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ACUTE GENERALIZED MILIARY TUBERCULOSIS *

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Since the first description of acute generalized miliary tuberculosis by Bayle [1] (1810) there has been controversy concerning the pathogenesis of this disease. The main question has been whether the generalized dissemination of foci throughout the various organs of the body originated from a softened caseous focus (Buhl [2]), a vascular focus (Weigert [3-7]), or from within the thoracic duct.

A critical review of the literature leaves the impression that the rôle of active extrapulmonary tuberculous processes in the production of the dissemination has been underestimated and too much stress laid upon the search for vascular foci.

This study has been undertaken in order to review my cases of acute generalized miliary tuberculosis and, in the light of advanced knowledge of tuberculosis, to correlate the pathogenesis, pathology and clinical findings.

MATERIAL

In 1656 consecutive autopsies on tuberculous persons, performed in a period of 8½ years, there were 297 cases (17.9 per cent) of acute generalized miliary tuberculosis. One hundred and ninety-one of the 297 cases showed varying degrees of chronic pulmonary tuberculosis in combination with the miliary dissemination; in 106 cases a generalized miliary seeding existed alone. Orth,[8] who found that more than half of his 30 cases of acute generalized miliary tuberculosis were associated with chronic pulmonary tuberculosis, is the only writer with whose findings my own coincide. Beginning with Weigert,[3] all other authors who discussed this relationship indicated their convictions that the presence of chronic pulmonary tuberculosis excludes the development of acute miliary tuberculosis. This "Ausschliessungsverhältnis" that the more extensive the chronic pulmonary tuberculosis the less the chance for the development of an acute miliary tuberculosis, has been accepted by Schmincke,[9] Huebschmann,[10] Liebermeister,[11] Schürhoff,[12] Schürmann,[13] Grethmann [14] and Pagel.[15,16]

I feel that the discrepancy between these findings and my own, results from the fact that these authors have interpreted the miliary foci found in lungs in conjunction with chronic pulmonary tuberculosis as representing bronchogenic spread from these chronic pulmonary lesions and have interpreted the miliary foci in the other organs as

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terminal hematogenous disseminations from the lungs. I feel that they have failed to recognize the combined picture.

General Considerations

There are a number of striking differences between the group of acute generalized miliary tuberculosis and the one in which this condition is combined with chronic pulmonary tuberculosis.

Age. From Table I it may be seen that in both groups the greatest number of cases is observed in the age period between 20 and 39 years. This does not agree with Grethmann [14] who has reported that youth and the older ages are chiefly involved, with the middle ages relatively free of miliary tuberculosis. Nor does it agree with Huebschmann [10]

Table I
Age Distribution

	Birth to 10 yrs.	11–19	20–29	30–39	40–49	50–59	60–69	Over 70
Acute generalized miliary tuberculosis	21	10	30	26	11	4	4	0
Acute generalized miliary tuberculosis and chronic pulmonary tuberculosis	11	10	46	48	35	30	10	1

and Hartwich [17] who emphasized the preponderance of acute generalized miliary tuberculosis in the first 2 decades. There is an interesting contrast in the early and late age distribution in both groups. There were 31 patients (29.2 per cent) who were less than 20 years of age in the series of uncombined acute generalized miliary tuberculosis and only 19 patients (17.9 per cent) who were older than 40 years. In the cases of acute generalized miliary tuberculosis combined with chronic pulmonary tuberculosis, on the other hand, there were 21 individuals (10.9 per cent) who were less than 20 years of age, whereas 76 (39.8 per cent) were more than 40 years of age.

This interesting contrast may in part be related to the source of the miliary tuberculosis. My studies have revealed that bone tuberculosis occurs chiefly in the younger age groups while the preponderance of male genital and urinary tuberculosis occurs in the older age groups. Bone tuberculosis was found in 48 of the 106 cases of acute generalized miliary tuberculosis; urogenital tuberculosis was found in 114 of the 191 cases of acute miliary tuberculosis combined with chronic pulmonary tuberculosis.

Color. In the 106 cases of uncombined acute generalized miliary tuberculosis 72 (67.9 per cent) were negroes, while 34 (32.1 per cent) were white. One hundred and twenty-two (63.9 per cent) of the 191

individuals showing acute generalized miliary tuberculosis combined with chronic pulmonary tuberculosis were white, while 67 (35.1 per cent) were negro and 2 (1 per cent) were of the yellow race.

Sex. An almost parallel distribution in respect to sex is present in the two groups. In the cases of uncombined acute generalized miliary tuberculosis, 73 (68.9 per cent) were males, while 33 (31.1 per cent) were females. In the other group, 147 (76.9 per cent) were males and 44 (23.1 per cent) were females.

Pathogenesis

Most of the discussion has centered upon the source of the hematogenous dissemination. Laënnec [18] (1810) was the first to point out that older soft caseous foci could lead to extensive dissemination throughout other organs. Buhl,[2] carrying his teachings farther, stated that in the majority of the cases of acute generalized miliary tuberculosis there were older caseous foci in the body. He suggested the resorption of the "specific substance" into the blood stream.

Huguenin [19] who shared Buhl's [2] view believed that the caseous mass could either erode and enter a small vein, or invade the lymphatics.

Weigert,[7] on the other hand, stated that, since this caseous focus is frequently not found, its occasional presence in cases of acute miliary tuberculosis is not sufficient evidence for belief in it as the source of the dissemination. He pointed to the infrequent occurrence of acute generalized miliary tuberculosis in conjunction with well developed phthisis. Since, he reasoned, in phthisis there are caseous processes and softened foci as well, and since these are apparently insufficient for the development of acute miliary tuberculosis, there must be other factors which allow the entry of masses of tubercle bacilli into the blood stream. In 1879 Weigert [6] observed a case of acute generalized miliary tuberculosis wherein a large caseous lymph node extended into the right innominate vein. In subsequent studies he found other venous tubercles in cases of acute generalized miliary tuberculosis. Accordingly, Weigert [3-6] contended that the vascular tubercle progresses from a tuberculous focus, which extends to the vessel's wall and after destroying its intima, ruptures into the blood stream. This focus is often referred to as the "Weigert" tubercle in spite of the fact that in one study he found vascular foci in only 50 per cent of the cases and in another in 70.8 per cent. This view of the vascular focus has been accepted by Pagel,[16, 20] Hartwich,[17] Schmorl,[21] Sigg,[22] Silbergleit,[23] Baar,[24] Dittrich,[25] Herxheimer,[26] Schwarz [27] and Berghammer.[28]

Benda,[29-31] likewise, believed that the vascular focus was responsible for acute generalized miliary tuberculosis. However, he considered that

the ulceration of a solitary metastasis within the intima rather than the erosion of the vessel wall by an extravascular focus was the source of the dissemination.

Ponfick [32] (1877) found that in the majority of his cases of acute generalized miliary tuberculosis he could demonstrate small tubercle-like nodules within the intima of the thoracic duct. It was his opinion that the tubercles in the thoracic duct were the result of the original hematogenous dissemination. Subsequently Schürhoff [12] and Grethmann,[14] in reviewing Ponfick's studies, stated that Ponfick did not recognize the tuberculosis of the thoracic duct as being primary and the generalized tuberculosis as being secondary.

Wild,[33] arguing against the views of Weigert, stated that there are too many cases (30 to 50 per cent) in which a focus cannot be found in the vein or thoracic duct. It was this author's opinion that small numbers of tubercle bacilli enter the blood stream from an active tuberculous process in the body (skeleton, urinary system, or other organ) and that the bacilli multiply in the blood stream due to certain favorable factors such as increasing virulence of the organism and special dispositional factors of the host. Ribbert [34, 35] shared Wild's views.

Huebschmann [10, 36, 37] rejected Weigert's views and stated that it is frequently impossible to isolate the source of the tubercle bacilli despite diligent search. He stipulated two factors leading to the generalization. The mechanical factor of a tubercle bacillus bacteremia (based on the findings of Loewenstein [38] and Liebermeister [39]) may be said to exist in all forms of tuberculosis. The dispositional factor depends upon (a) the specific component or lack of disseminated resistance in the body; and (b) the nonspecific component, or the unrelated metabolic and infectious processes of the host.

In a later study Loeschcke [40] (1931) postulated the development of acute generalized miliary tuberculosis as the result of a direct communication between a caseous focus and the blood stream without intermediate tubercle formation. Such a caseous focus, he pointed out, must contain large numbers of organisms, and through softening and liquefaction bacilli are released into the general circulation. Following this evacuation, blood enters the focus and can be readily detected microscopically. In a study of his cases of acute miliary tuberculosis which developed just before death he found grossly visible bleeding at the site of rupture with great regularity. The caseous focus which ruptured into the blood stream was usually a lymph node, but it was not infrequent to find it in the skeletal system, prostate, testis, or adrenals.

The findings of Loeschcke [40, 42] were subsequently substantiated by Minguez.[41]

A study of my own material does not allow me to accept any of the views of pathogenesis which are based on the assumption of the rupture of a tuberculous focus into the blood stream. It is my opinion that the development of acute generalized miliary tuberculosis is the result of the drainage of tubercle bacilli from an active extrapulmonary tuberculous focus (skeletal system, urogenital system, serous surfaces) or a primary focus (usually progressive) into the lymphatic system. This in turn empties into the venous system (through the well known lymphovenous routes).

It is interesting to note that Buhl [2] and Ponfick [32] alone have stated that the development of a generalized miliary tuberculosis may be the result of lymphatic drainage from a caseous focus.

The presence of an acute tuberculous process in one or more organ systems in every one of my cases of acute generalized miliary tuberculosis contradicts Huebschmann [10] whose observations led him to state that the two only occasionally occur together, and that there was probably an "excluding state" between the two conditions. Pagel [15] and Baar,[24] on the other hand, were not impressed by these "excluding" conditions, while Reisner [43] stated that the opposite seems to be the case.

It has been repeatedly stated that isolated organ-tuberculosis (third stage tuberculosis of Ranke, postprimary tuberculosis), in contrast to primary tuberculosis, is characterized by the absence of gross caseation of the lymph nodes. Although this is observed in the majority of cases of chronic pulmonary tuberculosis, the same may not be said of tuberculous involvement of other organ systems. It has been my repeated observation from autopsy material that progressive tuberculous involvement of the skeletal, urinary and genital systems is almost invariably characterized by caseous tuberculosis of the regional lymph nodes. The regional lymph nodes are also enlarged and caseous when there is a caseous process of the pleura, peritoneum and pericardium, and when there is extensive and deep-seated ulceration of the gastrointestinal tract. Unlike the primary complex the caseous involvement of the regional lymph nodes does not occur at the onset but usually develops in the later stages of the process. The enlargement and caseation of the lymph nodes is often not limited to the regional glands but may involve the neighboring chains.

The lymph node involvement indicates extensive lymphatic drainage of tubercle bacilli from the neighboring organ-tuberculosis, a phenomenon similar to that observed in any infection. This migration of

tubercle bacilli into the lymph nodes has been demonstrated by bacterial stains. The tubercle bacilli are carried in the lymph to the venous circulation and a generalized blood stream dissemination takes place with the development of miliary foci in the various organs of the body. Two important factors must be clarified in the consideration of a dissemination occurring in this manner: (1) caseation of regional lymph nodes does not necessarily mean that a blood stream dissemination will develop in every instance, just as it does not always develop from a pyogenic infection. The caseous process may be, and probably frequently is, walled-off with no resultant hematogenous dissemination. (2) After invasion of the blood stream takes place, the caseous involvement of the lymph nodes may become circumscribed by fibrous tissue. Repetition of the hematogenous dissemination is thus avoided and the hematogenous foci may undergo healing changes. Ample proof of this is seen at autopsy. It was observed most frequently in children in whom the generalization developed secondarily to a primary complex. These patients showed the caseous lymph nodes to contain calcium and to be encircled by a hyalinized connective tissue capsule; the tubercles in the various organs showed evidence of far advanced healing. Death, in these cases, was occasionally the result of tuberculous meningitis which developed from the generalization, but more often this cause was not related to tuberculosis.

Unlike the "Weigert tubercle," vascular perforation, or thoracic duct focus, the caseous lymph node is always present when there is a generalized miliary dissemination. It is difficult to imagine how caseous lymph nodes, laden as they are with tubercle bacilli, could exist without sooner or later pouring their contents into the general circulation via the efferent ducts.

Lymphatic drainage with subsequent hematogenous dissemination is observed in cases of chronic pulmonary tuberculosis (with or without gastrointestinal tuberculosis). The dissemination occurs most frequently into the spleen and kidney (liver is excluded because of possible portal drainage from gastrointestinal tuberculosis), but sometimes also into the adrenals and other organs including the brain. This hematogenous dissemination was observed in 33.2 per cent of the cases showing chronic pulmonary tuberculosis without associated extrapulmonary foci. In most instances caseous foci could be demonstrated within the tracheobronchial lymph nodes only on microscopic examination, but in some, particularly the more acute cases, the lymph nodes were grossly caseous. The hematogenous foci were more numerous and more widely disseminated in the individuals in whom the lymph nodes were grossly caseous.

Further propagation of the hematogenous dissemination occurs through the development of ulcerative tuberculous foci in the pulmonary veins. These foci develop in the walls of the veins (from within or without) in the course of the original seedings in the lungs. They undergo ulceration and the caseous contents laden with tubercle bacilli are carried to the left side of the heart and thence into the greater circulation.

Pathology

General Distribution

A true evaluation of the question of general distribution is beset by a number of difficulties. Whereas the lung readily lends itself to the gross identification of miliary tubercles, other organs are less productive on simple inspection. Easy recognition is of considerable importance, particularly when the dissemination is not extensive, since one millimeter foci may so readily be overlooked in routine examinations. In these instances, successful gross examination would be of immeasurable value. This is best exemplified in the kidney where division into 2 or 3 mm. slices will frequently reveal the presence of miliary tubercles overlooked in the routine examination. Such meticulous examination is of no aid in the heart, adrenals, pancreas, or thyroid gland because in these organs such foci are not recognizable on gross examination. A true incidence therefore, of the distribution within these organs can be ascertained only by serial section, a physically impossible task. The brain was removed for examination in only a small number of cases.

When the dissemination is extensive routine microscopic examination will reveal miliary tubercles in almost all parenchymatous organs.

Miliary foci were found in the lungs and spleen in all instances; in the kidney in 205 cases (69.0 per cent), in the adrenals in 84 cases (28.3 per cent), in the brain in 31 cases (10.4 per cent), in the pancreas in 14 cases (4.7 per cent), and in the thyroid in 10 cases (3.4 per cent).

Lungs

Clinical and Anatomic Correlations. The gross appearance, histologic characteristics and serial roentgenograms must all be considered in determining the development and ultimate fate of the miliary tubercle. Since all of the 297 patients in my series were in our institution, suffering from some form of extrapulmonary tuberculosis, an excellent opportunity was afforded to study the development and ultimate fate of the miliary dissemination. In this correlation only those cases were considered in which the lungs were clear before the hematogenous dissemination. The only proof of pulmonary seeding is the roentgeno-

gram; clinical signs and symptoms must be verified by x-ray examination. Huebschmann and Arnold,[44] in attempting to arrive at a classification of hematogenous seeding based on clinical signs and symptoms, came to the conclusion that there is a definite relationship between the clinical and anatomic pictures of generalized miliary tuberculosis; this, in spite of the fact that a number of their clinical observations did not coincide with the anatomic findings. Grethmann [14] and Pagel [15] emphasized the difficulty of correlating the age of the disease with the clinical data. None of the authors mentioned roentgenographic studies as correlation. I feel that this is a vital omission.

Some of the patients in my series whose earlier roentgenograms showed either clear fields or at most a few scattered foci in the apical portions, and who were observed over a period of months, developed diffuse disseminations in the lungs. Yet gross and microscopic studies revealed both recent and very old foci. The answer to this discrepancy lies in the fact that sufficiently early roentgenographic observations were not available. Occasionally, however, I have had the opportunity of observing the following course: a patient with some form of extrapulmonary tuberculosis has a roentgenogram which clearly demonstrates a diffuse miliary lung field dissemination. Subsequent studies then demonstrate progressive clearing from the caudal toward the apical aspects until, finally, no foci are visible or, at most, they are limited to the superior portions of the upper lobe. After a number of weeks or months another dissemination is observed and if the patient lives long enough another clearing may occur from below upward. Microscopic studies explain the apparent disappearance of the foci in the roentgenograms. One of the cases in my series had four separate seedings in the course of 15 months. These were demonstrated by x-ray and confirmed by microscopic examination which clearly demonstrated miliary lesions of four separate age groups. If this patient had been admitted to the hospital after the third dissemination had cleared roentgenologically, one would have had the mistaken impression that death had occurred after one seeding and that the foci were all of a recent date.

Not all individuals show clearing of the foci; some show progressively increasing numbers of miliary foci in subsequent roentgenograms —evidence of further disseminations. It has been my experience from serial roentgenographic studies, and from gross and microscopic examinations, that with the exception of those patients who die shortly after their first disseminations, the hematogenous seedings are usually multiple.

Gross Appearance, Distribution and Size. The dissemination in the

lungs is present from the apices to the bases. The foci are larger and more numerous in the apical parts and become smaller and less numerous toward the caudal aspect. They are larger and more numerous in the anterior than in posterior aspects of the individual lobes. Although most of the foci throughout the lung are approximately 1 mm. in size, those in the apico-ventral aspect of the upper lobe may measure 2 to 3 mm. and often have the appearance of acinous foci. Those in the posterior and basal portions of the lower lobes are almost invariably less than 1 mm. in size.

This variation in size and distribution has been variously interpreted by a number of authors. Buhl [2] and Ribbert [35] believed that miliary tuberculosis begins in the apices and progresses toward the bases so that the larger tubercles are the older ones and the smaller are the younger. Roentgenographic studies reveal the fallacy of this view. When dissemination occurs it is diffuse throughout the lung parenchyma. Schmorl [21] attributed the difference in size to factors which are more favorable for the rapid growth of tubercles in the upper parts of the upper lobe.

The answer to this question may lie in the posture of the lungs. Medlar and Sasano [45] injected tubercle bacilli intravenously into rabbits and strapped one group in an upright position for 10 hours daily and kept the other group in the normal position. They found that in the latter animals the tuberculous process developed chiefly in the posterior parts of the lung while those in the upright position had a preponderance of the lesions in the anterior areas. In both positions the lesions developed chiefly in the apical portions.

In a recent study [46] I pointed out the effect of mechanical compression of the lungs (thoracoplasty, artificial pneumothorax, empyema) on the dissemination of foci within the parenchyma. When dissemination occurred in these cases the noncompressed lungs contained the typical miliary seedings; the compressed lung showed none, or a markedly decreased number. Similar is the distribution of foci within lung parenchyma when chronic pulmonary tuberculosis precedes the seedings. The extent of this dissemination depends upon the amount of uninvolved lung parenchyma present before the hematogenous seeding occurs. The miliary foci appear in the previously clear lung tissue and the extent varies from the presence of seedings in all lobes to a distribution of foci limited to the lower part of one or both lower lobes. Foci in the upper lobes are larger than those in the lower lobes.

The most recent dissemination in my series was observed 5 days before death and the oldest dissemination occurred 15 months prior to autopsy examination. Thus the opportunity has been afforded to study

generalized miliary tuberculosis in all stages of development. Only for a limited period after the first dissemination do all the foci appear to be of the same age. In a short time they are of varying anatomic ages because of repeated seedings.

The foci in the earlier stages of development are yellow in appearance and fuse irregularly with a surrounding lung parenchyma which is firm and red. Although some of the tubercles are round or oval, most of them are irregular. In the later stages of development the tubercles are spherical, gray and well demarcated from a surrounding lung parenchyma which is resilient and often emphysematous. These foci represent the older seedings and are smaller than those in the earlier stages. Also usually present throughout the lung parenchyma are yellow foci (more recent disseminations).

Some of the cases in my series afforded the opportunity of studying far-advanced healing. The foci were either invisible to the naked eye or appeared as small gray or gray-black strands of tissue surrounded by emphysematous lung tissue. They were present chiefly in the apical portions of the upper lobes.

Microscopic Appearance. There has been much discussion as to whether the tubercles develop within the alveolar spaces (intra-alveolar) or in the interstitial tissue (interalveolar). My own findings substantiate the former contention. The first change is that of an alveolar filling process composed of polymorphonuclear leukocytes, alveolar phagocytes, occasional red blood cells and variable amounts of fibrin. In many instances fibrin is entirely absent. The alveoli thus involved are from two to five in number. A few of the surrounding alveoli contain alveolar phagocytes, lymphocytes and occasional red blood cells. The capillaries in the interstitial tissue here are dilated and filled with blood; this is the perifocal reaction.

As the process continues the area of pneumonia may either (1) undergo caseation or (2) be invaded by productive elements. (1) In the first instance the exudate within the alveoli and the fixed tissue elements undergo caseation and with the hematoxylin and eosin stain the focus has a pink-blue granular, and later a pink, appearance. The elastica and van Gieson's stain reveals that the elastic fibers of the alveolar septa, blood vessels and bronchioles within the zone of caseation are still intact. Surrounding the area of caseation is a zone of fibroblasts, epithelioid cells, giant cells and collagen fibrils. I have noted that exudate containing fibrin has a greater tendency to undergo caseation. (2) In this case fibroblasts, epithelioid cells, giant cells and collagen fibrils invade the focus. In the earlier stages the alveolar structure is well retained and the productive elements are intermingled

with the exudative components in the alveolar spaces and are present within the walls of the alveolar septa. As the process continues these productive elements within the alveoli replace the exudate and destroy the septal walls. The end result is an oval or round tubercle in which the underlying lung architecture is completely destroyed.

Healing is observed within the miliary tubercles in both types of foci. The peripherally placed productive elements of a focus with a central zone of caseation extend into this caseous center and gradually replace it. As the process continues, the collagen fibrils formed by the epithelioid cells and fibroblasts increase in number and fuse. With this progression a concomitant decrease in the cellular elements results until finally only a concentric zone of hyalinized connective tissue remains. A focus of the cellular type, in its progression to healing, shows an increase and fusion of the collagen fibers with again a disappearance of the cellular elements with the final formation of a concentric zone of hyalinized connective tissue. Subsequently these areas of connective tissue lose their concentric arrangement, become flattened and, with the disappearance of the specific elements, the resultant scars cannot be differentiated from those caused by other inflammatory processes.

As these cellular foci are transformed into hyaline connective tissue they gradually decrease in size. The surrounding alveolar septa which are intimately adherent to them become stretched and, as the tubercles become smaller, tear. This results in alveolar dilatation and even bleb formation around the healing and healed tubercles. The hyperventilation of the areas encircling these healing tubercles and the diminution in the size of the foci themselves cause a partial or complete disappearance of the characteristic x-ray picture of miliary seeding. Since the foci in the basal areas are smaller and less numerous, the fading of the foci from the roentgenogram is noted first in this portion. As healing progresses there is a progressive "resolution" toward the apical region, and if the source of the hematogenous dissemination has been shut off, all the foci disappear permanently from the roentgenogram. The foci, on gross examination, are visible to the naked eye for a long time after they have disappeared from the x-ray field. Very often, however, a new dissemination appears before the previous seeding has completely "resolved" roentgenographically.

Spleen

Gross. With the exception of those cases in which the dissemination is extensive, miliary tubercles are not distinguishable from splenic corpuscles. When perceptible, they appear as gray elevated nodules which stand out above the cut surface as fine granules. They are approxi-

mately 1 mm. in size and in some regions there are larger foci which have a yellow appearance. I cannot agree with Huebschmann [10] that only the inexperienced cannot distinguish tubercles from the splenic corpuscles, the follicles being gray and the tubercles white.

Microscopic Examination. Although sometimes seen in the splenic corpuscles, tuberculous foci are usually present in the splenic pulp. This is in contrast to the findings of Gråberg,[47] Heitzmann [48] and Lubarsch [49] who found tubercles predominantly in the follicles. My observations as to the development of the tubercle agree with those of Huebschmann and Arnold,[44] Baumgarten,[50] and Schleussing.[51] The earliest changes observed were those of tissue damage. (Tubercle bacilli are numerous and easily demonstrated in the paraffin section at this stage.) This tissue damage is characterized by focal areas of necrosis in which numerous nuclear remnants give the area a blue, granular appearance with the hematoxylin and eosin stain. The underlying splenic architecture is still discernible within the focus in this stage of development. Polymorphonuclear leukocytes are always present and sometimes, in addition, there are occasional epithelioid cells. The question arises as to whether the stage of primary tissue damage is followed by an exudative phase characterized by the presence of polymorphonuclear leukocytes and fibrin and followed in turn by the productive reaction, or whether it is followed directly by the productive reaction. The difficulty in answering this question lies in the fact that most of the foci when first seen are too far advanced to make discriminating observations.

I tend to agree with Huebschmann [10] who felt that the exudative reaction (polymorphonuclear leukocytes and sometimes fibrin) may either go on to caseation or be followed directly by the invasion of the productive elements. If caseation should develop it is encircled by a narrow zone of epithelioid cells, fibroblasts with occasional collagen fibrils and giant cells. Within the caseated area in the earlier stages nuclear remnants and occasional polymorphonuclear leukocytes are present, an indication that the stage of caseation follows the exudative phase. More frequently have I observed the direct invasion and replacement of the exudative elements by the productive components. The result is the formation of an epithelioid-giant cell tubercle which is oval or round. With the invasion of the productive elements the underlying architecture is rendered indiscernible. Thus the spleen in the fully developed stage is studded with oval and round epithelioid-giant cell tubercles, some of the foci containing central regions of caseation. I have not been convinced that caseation ever follows the productive stage.

Healing of the tubercles occurs under the same circumstances and in the same manner as in the lungs. With the complete hyalinization of the foci all signs of specificity disappear and the origin of the connective tissue cannot be determined.

Kidney

In a generalized miliary dissemination one would expect to find miliary tubercles in 100 per cent of the cases. I believe that the reason why 34.1 per cent of cases are negative lies in the technical difficulty in thoroughly examining the kidneys for tubercles. The negative cases include those in which healing of miliary foci was found in other organs and those in which miliary dissemination was not extensive in the spleen or lungs. I think that my search was sufficiently complete to determine definitely the absence of foci. A thorough search would necessitate sectioning the kidney into 2 or 3 mm. slices, followed by serial microscopic study if no foci were found by such detailed gross examination.

Gross. In cases of diffuse generalized dissemination numerous foci are visible on the surface of the kidney when the capsule is removed. These foci appear as flat yellow areas, usually from 1 to 2 mm. in size, which fuse irregularly with the surrounding renal parenchyma. In the earlier stages a narrow red zone surrounds the miliary tubercles. Upon the cut surface the foci are frequently seen as linear areas which lie parallel to the radial rays. They are most frequent in the corticomedullary region, less common in the cortex and least numerous in the medulla.

Microscopic Appearance. The development and ultimate fate of the tubercle is similar to that in the spleen.

Adrenal Gland

Although the adrenal glands showed tuberculous involvement in 98 cases, 84 (28.2 per cent) actually contained miliary tubercles. The other cases presented large caseous foci which often caused marked enlargement of the gland. The anatomic picture of these latter foci clearly indicated that their development preceded the miliary dissemination into the other organs. In a number of cases such nodular caseous tuberculosis was limited to one side, and the miliary involvement of the opposite adrenal was similar to that seen in the spleen and kidneys.

Gross. Miliary tubercles are rarely visible in the adrenal gland on gross examination. When they can be seen, they appear as relatively few gray or gray-yellow foci present mainly in the cortex.

Microscopic Appearance. The appearance of miliary tubercles of the adrenal is similar to those of the spleen and kidney. They are most frequently found in the zona fascicularis of the cortex and are usually not numerous.

Conclusions

As a result of a pathologic analysis of 297 cases of acute generalized miliary tuberculosis, a revised concept of the pathogenesis of the condition is presented for consideration. The invariable presence of caseous lymph nodes in these cases and the analogous situation to that in other infectious diseases lead to the conclusion that the lymphovenous circulation provides the mechanism for miliary dissemination.

The correlation of several roentgenographs with the anatomic appearance of the miliary foci in each patient demonstrated that a hematogenous dissemination of tubercle bacilli sufficient to produce generalized miliary foci is not necessarily an incurable condition. Such correlation demonstrates the almost invariable occurrence of multiple seedings, some or all of which may resolve.

The usual concept, that the concomitant presence of chronic pulmonary tuberculosis and acute generalized miliary tuberculosis is very rare, is not confirmed by this study. This combination occurred in 64.3 per cent of the cases in the series upon which this paper is based.

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CONGENITAL ABSENCE OF THE PERICARDIUM

Report of A Case *

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Absence, or deficiency of the pericardium is one of the rarer of the congenital malformations. Historically, it is of interest to note that the first case of absence of the pericardium was described by Realdus Columbus in 1559, though this condition was first accurately described by Baillie in 1788. Since then, reports of individual cases have been made occasionally in the literature. The present case brings the total number reported to 74.

This condition, aside from its rarity, possesses through the cases reported a special interest in attempting to throw some light on the function of the pericardium, a subject still presenting a diversity of opinions. The function usually assigned to the pericardium because of its position and relations has been a restraining influence against cardiac overdilatation, a view not well supported by animal experimentation or by observation of human cases in which there was a congenital deficiency. But its presence in all mammalian hearts would certainly imply some functional rôle. Eyster, in 1931, suggested that it has a restraining influence against that degree of dilatation which in long-continued activity would lead to hypertrophy and subsequent degeneration. Beck, in 1931, in an analysis of the reported cases of deficient pericardium, concluded from these studies that there is no evidence to indicate that the pericardium exerts a beneficial effect on the acutely dilating heart and may even at times constitute a grave danger to the mechanics of the circulation, particularly in those cases in which cardio-pericardial adhesions develop.

Watt (1931), in presenting a case of a rare congenital deficiency of the pericardium and in a review of an extensive bibliography, concluded that this condition is not incompatible with a long, active life, free from any special weakness of the heart, and that a separate pericardium is not absolutely essential to the normal functioning of the heart. A secondary function of the pericardium is to provide a nearly frictionless serous sac to facilitate the movement of the heart. This function can be accomplished quite well with the heart in the pleural cavity.

From the developmental viewpoint, a defect in the pericardium may be due to a premature atrophy of the left duct of Cuvier, resulting

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from some anomaly of the circulation of the great venous trunks. This anomaly leads to an arrested development of the pulmonary ridge, resulting in a localized defect of the pleuropericardial septum. This defect would occur in the fifth week of fetal life when the pericardial column begins to separate from the pleural column.

Report of Case

The patient was a young, adult, white male soldier, 27 years of age, who was 71½ inches tall and weighed approximately 170 lbs. His bony frame was moderately heavy and muscular development good. He was admitted to the hospital with a perforating gunshot wound. The point of entrance was in the nipple line at the level of the fifth intercostal space on the left side of the chest anteriorly. The bullet traversed diaphragm, spleen, and left lower lobe of the lung. Operation consisted of excision and closure of chest wounds and laparotomy. As both respiratory and circulatory collapse were imminent, the failing heart was reached for through a lower perforation in the diaphragm and gently clasped in the hand for the purpose of manual stimulation. At this time it was noted that the heart was free of any pericardial covering and contained in the same serous cavity as the left lung, a condition signifying the absence of a pericardium, which was thus recognized during life.

Autopsy Findings

Necropsy confirmed the observations made at the time of the operation, with the following pertinent findings:

The mediastinum was seen to be in approximately normal position. The anterior surface of the parietal pericardium was absent. Further exposure disclosed a portion of the membrane over the ascending aorta anteriorly, through a distance 3.0 cm. vertically downward from a normal point of attachment at the beginning of the transverse portion of the arch. This portion of the pericardial membrane showed a rounded, smooth, grayish pink edge and continued from a point 3.0 cm. above the base of the aorta downward and to the right, joining the mediastinal tissue and pleural surface at approximately the level of the base of the aorta. Several small, grayish tissue bands were seen between the mediastinal wall and the base of the heart.

Close examination of the medial portion of the afore-mentioned pericardial membrane showed a continuous edge, visible through a few thin connective tissue bands, extending downward parallel and in contact with the surrounding fatty tissue. The membranous edge circled beneath the inferior portion of the base of the heart and continued as a free edge of a second portion of the pericardium at the left inferior side of the base of the heart. This second portion of pericardium measured 5.0 cm. along the free edge which extended from the lower edge of the cardiac base to a point 3.0 cm. below the left auricular appendage. Between this point and the lower edge of the auricular appendage, the membrane appeared to fuse with the visceral pericardium along the

atrioventricular groove. The free edge was slightly concave. The membrane measured 5.0 cm. at the broadest portion, forming a pouch with smooth inner surface. The external surface, between the free edge and the left pulmonary ligament, showed small lobular masses of fatty tissue.

The upper left side of the heart near the base showed a thin, fenestrated membrane extending from the left inferior auricular surface to the corresponding portion of the medial surface of the upper left lobe.

The medial and inferior anterior borders of the heart were covered by a fatty membrane extending from the interior pleural-mediastinal and pleural-diaphragmatic edge, forming a bed of fatty tissue conforming to the shape of the heart. The membrane extending from the anterior mediastinal edge measured 0.5 to 1.0 cm. in thickness and 2.0 to 4.0 cm. in width, and the one from the anterior diaphragmatic edge measured 1.0 to 3.0 cm. The surface of the fatty tissue was lobular, soft, yellow and slightly translucent, without evidence of a fibrous membrane.

The heart weighed approximately 350 gm. The epicardium over the entire anterior surface was faintly grayish and opaque. A more definite area of gray opacity, seen at the anterior surface near the apex and corresponding to the most anterior portion of the wall, measured 4.0 by 2.0 cm. Scattered subepicardial hemorrhages were seen over the posterior surface, the largest 1.0 cm. in diameter. The great vessels and mediastinal structures appeared to be in normal relation. The thymic area was occupied by fatty tissue.

Summary

1. A case of congenital absence of the pericardium is described which was recognized during life and confirmed by autopsy.

2. The condition is compatible with an active, strenuous life.

3. A separate pericardium is not essential to the normal functioning of the heart. The function of a frictionless serous sac to facilitate the movement of the heart can be taken over by the pleural cavity.

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DESCRIPTION OF PLATE

PLATE 26

FIG. 1. The heart is shown with retraction of the medial fatty membrane to the right exposing the edge of the pericardial remnant. (Photograph by Signal Corps, U. S. Army.)

FIG. 2. Left anterior view of the base of the heart with the apex of the heart retracted to the right. Arrow points to the edge of the pericardial remnant which continues to the right under the base of the heart. (Photograph by Signal Corps, U. S. Army.)

2

1

CARCINOID TUMOR OF THE CECUM WITH METASTASIS *

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Carcinoid tumor of the appendix is a familiar entity to pathologists and to many surgeons, but the occurrence of carcinoids elsewhere in the gastrointestinal tract appears, from a review of the pertinent literature, to be relatively uncommon. The reported cases of carcinoid tumors of the cecum, particularly, are rare. Stout,[1] in a recent article, reported the distribution of carcinoid tumors of the large bowel and found only 4 occurring in the cecum, all of which he designated as malignant. Mayo and Wilson,[2] reporting a metastasizing carcinoid of the cecum, found only 3 other carcinoids of the large bowel, all of which had metastasized. Wyatt [3] reported 3 carcinoids, 2 from the appendix and 1 from the cecum. The latter had metastasized to the liver.

From an examination of the literature there are found summaries of the reported cases of carcinoids compiled by Forbus,[4] Cooke,[5] Ariel,[6] and Dangremond.[7] The total number of reported cases is 283, distributed throughout the gastrointestinal tract from the stomach to the rectum. The most common site is in the vermiform appendix, and approximately 85 per cent of all carcinoids occur in the ileocecal region including the appendix and the ileum. Occurrence in the remainder of the small bowel is less frequent. Stout [1] reported 10 cases which were found in the colon and 12 which occurred in the rectum; the latter group includes 6 rectal tumors in that author's series, 5 of which differed cytologically from the ordinary carcinoid. Isolated cases have been described as occurring in the stomach and in a Meckel's diverticulum.

Raiford [8] reported the incidence of carcinoids in the Pathology Laboratory of the Johns Hopkins Hospital. He found 29 cases among 62,000 specimens, including autopsy and surgical material.

We wish to add the following case of carcinoid of the cecum to the 4 so far reported. In common with these, our case has local and distant metastases.

REPORT OF CASE

The patient, an elderly white male, 72 years of age, had been seen in the outpatient clinic for several years because of vague complaints referable to the gastrointestinal tract. He had been studied thoroughly, including gastrointestinal x-rays and extensive laboratory procedures, but a positive diagnosis was not obtained. He was admitted to the hospital 4 months before death, at which time he again complained of gastrointestinal disorders. The principal physical finding at this time

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was tenderness and rigidity in the right upper quadrant and the abdomen, with a mass which was thought to be an enlarged liver. It was felt that the patient probably had a carcinoma of the gastrointestinal tract which had escaped diagnosis, and a peritoneoscopy was done. Multiple nodules in the liver were noted, the largest estimated to be 4 by 6 cm. These were yellow-white in color and appeared to be neoplastic, but there was no evidence of carcinoma elsewhere. Therefore the diagnosis was made of metastatic carcinoma of the liver, primary site undetermined. The patient had a progressive downhill course and expired.

Necropsy

Gross Examination. The examination of the thorax was irrelevant. The peritoneum was shining, smooth and glistening. The liver weighed 1480 gm. The color was basically deep purple-brown, but the surface was studded with yellow nodules averaging 5 cm. in diameter. Most of these nodules were spherical and were much firmer than the surrounding liver parenchyma. The surface made by cutting showed these nodules to be uniform in color and consistency throughout, and sections through the liver showed the distribution of these nodules to be general throughout all lobes. The cecum was purple to red and slightly distended. The appendix was retrocecal and curved upward and inward and was partly obscured by an adherent spherical mass 2.4 cm. in diameter which appeared to arise from the serosa on the medial aspect of the cecum 8 cm. above the ileocecal valve. It had no connection with the appendix. The surface of this nodule made by cutting was smooth and was yellow to brown. Connected with this mass was a white cord which was firm and approximately 0.3 cm. in diameter and which extended medially. At the point of junction of this cord with the mesentery were many firm, discrete lymph nodes 0.5 to 1.5 cm. in diameter which were yellow to brown. The mucosa of the entire colon and of the small bowel was entirely normal. The examination of the remainder of the abdominal contents showed nothing of importance.

Microscopic Examination. Sections of the cecal tumor showed closely packed groups of cells, which for the most part were polyhedral and round. The nuclei which were oval were deeply stained, and the outline of the cytoplasm was somewhat indistinct. There were some granules in the cytoplasm. Around the borders of these nests there was a tendency toward a palisade arrangement, and these cells had the same nuclear pattern, but they were somewhat columnar in shape. Some cells were arranged in a circular pattern around a cavity or a vesicle and formed a rosette. In these the nucleus was basally placed and the major portion of the cytoplasm was centrally placed toward the vesicle. The individual nests of cells had a fine connective tissue stroma and were separated from each other by coarse fibrous connective tissue bands. Sections of the lymph nodes showed complete replacement of the lymphoid tissue by cells similar to those described. The tumor cells were in smaller nests, and the fibrous connective tissue was

more abundant and of a coarser variety. The rosette pattern was absent. Sections of the liver showed extensive invasion by the same type of tumor. The clumps or nests of cells resembled those found in the lymph nodes. They were uniformly smaller than those in the primary lesion and were separated by dense and coarse connective tissue bands.

Argentaffine granules were demonstrated in the cells of the cecal tumor and in those of the lymphatic and hepatic metastases after the method of Masson.[9]

Discussion

A review of the origin and development of these tumors readily accounts for their sites of predilection. Masson [9] stated that the structural features of carcinoids are sufficiently uniform, regardless of their location in the alimentary tract, to be grouped under the same title. "From their structure carcinoids were at first thought to be carcinomata; .... Their usual benignity and their small size earned for them the name little carcinomata (Lubarsch), then carcinoid (Oberndorfer) ...." Recent reports of metastasizing carcinoids make pertinent the question as to whether they should again be considered as carcinomata.

Carcinoids are composed of epithelial cells which are grouped in nests and in columns. Three different types of cells are found: (1) round or polyhedral, (2) palisade cells which are columnar, and (3) prismatic cells which form rosettes around a cavity or a vesicle. All of these cell types may appear in a single nest together with intermediary forms.

According to Masson,[9] the final histologic diagnosis of a carcinoid depends on the identification of argentaffine granules in the protoplasm. Stout [1] has described carcinoid tumors in the rectum composed of the pre-enterochrome cells of Erspamer. These differ cytologically from carcinoids of the usual type.

The recognition of carcinoids is often incidental at autopsy. Many of these tumors are small and produce no symptoms. Others occurring in the small intestine produce symptoms of intestinal obstruction which are usually the result of a kinking or intussusception rather than of actual constriction such as is seen in carcinoma. Other clinical findings may be diarrhea, anorexia, loss of weight, abdominal pain and weakness. Melena is rarely encountered since these tumors rarely ulcerate.

It is generally considered that carcinoids are benign neoplasms since many which occur in the appendix remain entirely localized. However, a considerable number have been described with metastases to the mesentery, regional lymph nodes, lungs, liver, spleen, adrenals, kidney, brain and bone marrow. Cooke [5] emphasized the potential malignant character of carcinoids and included 21 metastasizing tumors of this nature in his series.

Mayo and Wilson [2] recently reported their case as adenocarcinoma grade II of the "carcinoid" type with involvement of the regional lymph nodes, and Wyatt [3] stated that all carcinoids must be considered to be slowly growing malignant tumors. From the consideration of the case herein reported, it appears that the benign nature of carcinoids has been somewhat overemphasized.

We find some difficulty in explaining the occurrence of the tumor in our case outside the serosa of the cecum, although the tumor reported by Mayo and Wilson [2] appears to have been similarly situated.

Summary

A fifth case is added to the reported series of carcinoid tumors of the cecum.

In common with those previously reported, our case had local and distant metastases.

It is suggested that the term "carcinoma of the carcinoid type" be substituted for the term "carcinoid."

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DESCRIPTION OF PLATE

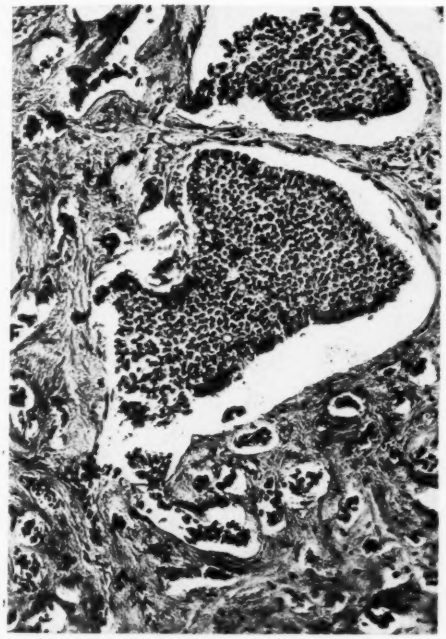
Plate 27

Fig. 1. Section through the cecal tumor. All three cell types are found in the larger nests: round and polyhedral, columnar, and rosette types. The fibrous partitions are coarse. Masson's trichrome stain. × 129.

Fig. 2. Higher magnification of a field in Figure 1 showing the palisade arrangement and the rosette formation. Masson's trichrome stain. × 515.

Fig. 3. Section through a lymph node showing complete replacement of the lymphoid tissue by nests of carcinoid cells and connective tissue stroma. Masson's trichrome stain. × 64.

Fig. 4. Section through the liver showing invasion by many small nests of carcinoid cells. Masson's trichrome stain. × 64.



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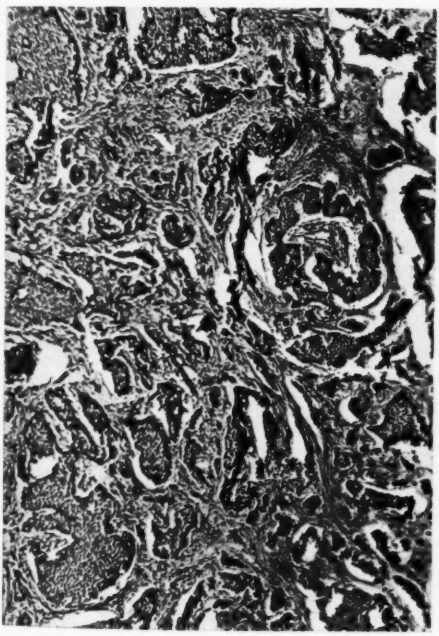

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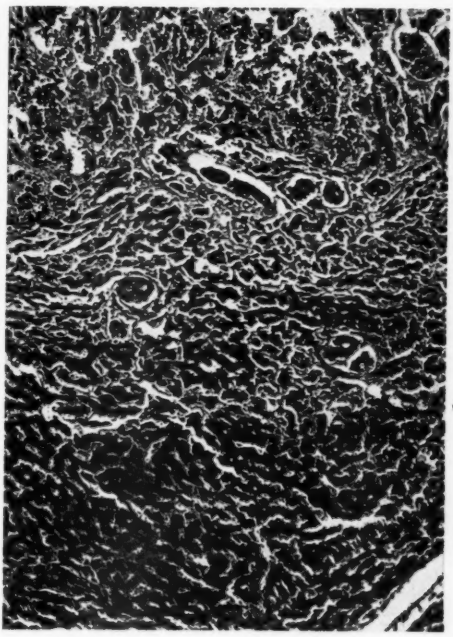

4

Potter and Docter

Carcinoid of the Cecum

CEROID, THE PIGMENT OF DIETARY CIRRHOSIS OF RATS

Its Characteristics and Its Differentiation from Hemofuscin *

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A peculiar pigment occurring in rats fed choline-deficient diets has been reported from several laboratories. Lillie, Daft, and Sebrell [1] described a coarsely globular pigment which occurred in phagocytes in the cirrhotic livers of rats fed low protein (4 per cent), low fat (5 per cent) diets. In subsequent papers [2-4] they reported its occurrence in liver cells, lung, spleen, lymph nodes, bone marrow and adrenal cortex. They were able to prevent its production by supplementing the basal diet with choline, methionine, and casein singly or in combination. They named the pigment "ceroid" [4] because of its wax-like appearance and behavior. Blumberg and McCollum [5] and Blumberg and Grady [6] reported a similar pigment in the cirrhotic livers of rats fed low protein, high fat diets. Edwards and White [7] reported a similar pigment in the livers of rats fed low protein diets supplemented with the carcinogen, p-dimethylamino-azobenzene (butter yellow). In a subsequent paper, White and Edwards [8] reported the pigment in rats fed the basal diet without butter yellow. Smith, Lillie and Stohlman [9] produced cirrhosis but no ceroid in rats fed high protein diets and butter yellow. György and Goldblatt [10] found a similar pigment in the cirrhotic livers of rats fed low protein, high fat diets.

In view of the specific occurrence, peculiar properties and possible metabolic significance of this pigment (henceforth referred to as ceroid), it seems proper to characterize it more fully. The following observations were made in this laboratory upon liver fixed in formaldehyde and embedded in paraffin.

(1) As previously reported,[4] ceroid occurs as globules varying in diameter from 1 to 20 μ. These are seen occasionally in liver cells but ordinarily in large rounded phagocytes. The phagocytes usually contain several globules and are seen in greatest number in the liver. In minimal cases a few phagocytes are seen between liver cell cords or surrounding central veins, or rarely in portal areas. In moderate to marked cases, numerous phagocytes form broad sheets and trabeculae which divide and encircle nodules of liver cells. Ceroid has been seen in phagocytes in alveolar septa of the lung, in splenic pulp, bone marrow, lymph nodes and adrenal cortex. A substance with similar staining re-

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actions occurs as rims or halos about large fat vacuoles in the liver. The large vacuoles are presumed to lie in liver cells.

(2) In sections stained in hematoxylin and eosin, van Gieson's connective tissue stain, Masson's trichrome stain, and in sections immersed for 1 hour in 1/1000 aqueous acid dyes: naphthol green B, orange G, congo red, trypan blue, tartrazine [C.I. no. 640], acid fuchsin, eosin Y, eosin B, erythrosin bluish, phloxine B, rose bengal, nigrosin W.S., alizarin rubinol [C.I. no. 1091], alizarin saphirol [C.I. no. 1054], indigc carmine, and orcein, and in paraffin or celloidin sections mounted unstained, the ceroid appears as pale yellow globules.

(3) Eosin and polychrome methylene blue stain ceroid yellowish green to greenish blue. It is stained slowly by a number of basic dyes when immersed for 1 hour in 1/1000 aqueous solutions. The following dyes all stained ceroid: Bismarck brown, malachite green, brilliant green, pararosanilin, basic fuchsin, new fuchsin, methyl violet, crystal violet, methyl green, safranin O and D, methylene violet RRA, brilliant cresyl blue, Nile blue A, methylene blue, toluidine blue, and cresyl violet. The slowness with which ceroid is stained by basic aniline dyes may explain Edward and White's[7] failure to stain it in fuchsin and in phloxine methylene blue.

(4) It is strongly acid-fast. When stained with steaming Ziehl's carbolfuchsin for 10 minutes, the bright red globules resist decolorization in 3 per cent hydrochloric acid in 70 per cent alcohol for at least 2 days.

(5) It gives several reactions for fat. It stains brownish orange to orange-red with sudan IV in frozen sections. With sudan brown, it stains brownish orange. In paraffin sections the sudanophilia is retained provided the sections are mounted in the usual fat-stain mounting media. In the Lorrain Smith Nile blue sulfate stain for differentiating neutral fats, many ceroid globules stain reddish purple while a few are blue, but if such sections are mounted in xylol clarite, most of the globules turn blue in a day or two (paraffin sections). In frozen sections, some are blue while most are reddish purple. Ceroid is black after treatment of paraffin sections or formaldehyde-fixed blocks with osmic acid, with or without antecedent chromation.

(6) It is stained red in Mallory's stain for hemofuscin. In this connection it was noted that 1/1000 aqueous basic fuchsin failed to stain ceroid in 5 minutes, stained a few globules in 10 minutes and stained almost all globules in 30 minutes.

(7) It is negative for iron with both the acid ferrocyanide test of Perls and the ammonium sulfide-Turnbull blue method.[11]

(8) Ceroid is gram-negative in Weigert's stain for fibrin and bac-

teria, in Lillie's [12] acetone technic, and in Lillie's [13] thiosulfate technic. When stained for 2 hours in hot crystal violet and then treated with iodine and acetone, ceroid stains violet rather than the blue-black of gram-positive bacteria.

(9) It stains gray to black in Weil's modification of Weigert's stain for myelin.

(10) It reduces silver nitrate very slowly and irregularly. Foot's [14] diamino-silver carbonate solution is reduced in 48 to 96 hours, giving brown to black ceroid globules.

(11) In paraffin sections, ceroid does not reduce ferric chloride-ferricyanide in Schmorl's [15] test for "Abnutzungspigmente."

(12) It is stained a light brown by Gram's iodine.

(13) In ultraviolet light under the fluorescence microscope, the globules of ceroid display a greenish yellow fluorescence which changes slowly to a yellowish white fluorescence. The fluorescence is displayed by frozen sections and by paraffin sections dry, in water, or in paraffin, but not in xylol or xylol clarite.

(14) It is not removed from paraffin sections by prolonged treatment in water, alcohols (methyl, ethyl, propyl, or isopropyl), acetone, ether, chloroform, benzene, xylene, gasoline, propylene glycol, hydrogen peroxide, chlorine water, potassium permanganate, dilute acids (hydrochloric, sulfuric, acetic, nitric), or dilute alkalies (sodium hydroxide, sodium carbonate, ammonium hydroxide).

(15) In autolyzed material, where basophilia is poor, this staining quality of ceroid is improved by post-formaldehyde block mordanting with picric acid, or in paraffin sections by treating with potassium permanganate followed by oxalic acid, by treating with picric acid, or by treating with iodine followed by thiosulfate.

(16) When a formaldehyde-fixed liver is ground with mortar and pestle, suspended in distilled water and allowed to stand overnight, the ceroid settles out in a sharply defined layer of bronze-brown viscid material just above the liver débris from which it may be separated in a separatory funnel. When extracted with chloroform, ether and acetone and evaporated to dryness this material yields a coarsely granular, dark brown residue. The residue, when crushed on a slide in a drop of water and examined microscopically, is seen to consist of ceroid globules and a little cellular débris. This crude concentrate reacts to stains and solvents in the same manner as ceroid in paraffin sections. It is decomposed by concentrated mineral acids and strong alkalies. It chars but does not melt on heating. It gives a negative Gmelin reaction and produces no color reaction in the chloroform-sulfuric acid test for carotenoids. It is slowly dissolved in boiling 10 per cent sodium hydroxide

from which a fatty substance is precipitated on neutralization with hydrochloric acid.

Discussion

The chemical nature of ceroid cannot be fully stated at present. Several theories as to its composition have been advanced by other laboratories and will be discussed briefly.

Blumberg and Grady [6] regarded the pigment as hemofuscin. However, von Recklinghausen,[16] who first described hemofuscin, characterized it as a gall brown, very finely granular, iron negative pigment which occurs in hemochromatosis along with large amounts of hemosiderin. He found it in smooth muscle of the intestines and blood vessels, in mast or connective tissue cells of the vascular supporting tissue (Glisson's capsule, etc.) and in certain cells of the salivary and lacrimal glands. Hemofuscin, as previously described and as further characterized by Mallory, Parker and Nye,[17] has been studied in this laboratory in material from cases of hemochromatosis, some of which was furnished us by the Curator of the Army Medical Museum. It was found to differ from ceroid in appearance, location and staining. It is not acid-fast, stains readily with dilute basic aniline dyes and is not sudanophilic after paraffin embedding.

Edwards and White [7] suggested that it may be a conjugated lipoid. György and Goldblatt [10] advanced the theory that it may represent a lipoid conjugated with a protein and may arise from necrotic remnants of liver cells. Available evidence lends some support to these theories. Ceroid gives several reactions for fat. It is not, however, soluble in ordinary fat solvents and, furthermore, demonstrates staining reactions not shared by neutral fat, ordinary fatty acids and the simpler lipoids.

In this laboratory it has been observed in cases of progressive cirrhosis as small globules within apparently surviving and often fat-free liver cells, suggesting that it may be a product of altered liver cell metabolism, but it also occurs as acid-fast, basophile rims about obvious coarse fat globules probably located in distended liver cells, suggesting origin also from what, in earlier stages of the process, appears to be neutral fat. Similar acid-fast material originating from foreign fat has been reported in cod liver oil pneumonia,[18, 19] but the further agreement of this material with ceroid has not been fully established.

Ceroid has shown no properties which would justify classing it with anthocyanins, sterols, carotenoids, porphyrins, or the hemoglobin derivatives.

Material furnished this laboratory by Blumberg and by Goldblatt contains a pigment indistinguishable from the ceroid in our own material.

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FURTHER STUDIES ON THE PREGLOMERULAR CELLULAR APPARATUS *

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When, in 1927, I described the existence of a preglomerular cellular apparatus in the human kidney, I pointed out three main facts: (1) the constant presence of special cells in the afferent arterioles of the normal kidney and their principal morphological characters, especially their fuchsinophilic granulations; (2) their identity with the so-called neuromuscular cells in the vascular glomera as described by Masson; (3) their possible relationship to the mechanism of hypertension. Since then this cellular apparatus has been studied in numerous publications and the various views expressed on this subject as well as some new developments concerning the significance of these cells have been reviewed recently by H. W. Smith in his Harvey Lecture on renal circulation.

Upon reading this study one cannot but be impressed by a series of conflicting statements concerning this cellular apparatus. The existence of cells, which is affirmed by some authors, is denied by others, and often one has the impression that statements referring to certain structures are implicitly applied to others for which they have never been claimed. The main reason for this confusion is that, curious as it may be, most of the authors, including myself, who have first studied these cells were unaware of previous publications concerning the same or similar elements. Different technics were applied which did not always give the same results. Analogies were established which, upon closer examination, proved to be far from obvious and, finally, theories were advanced which from the beginning lacked all material proof. The picture thus created appears rather confusing.

The principal facts may be summarized as follows. Ruyter first called attention to structural peculiarities of the afferent glomerular artery characterized by a disappearance of the internal elastic membrane and by a thickening of the muscular layer due to the presence of granular cells. These cells seem to originate from muscular elements which in successive states of transformation change the shape of their nuclei, lose their fibrils and acquire acidophilic granulations. Ruyter studied these cells in mice; he was able to see them also in rats but he stated explicitly that he could not find them in the kidneys of man, monkey, dog, cat, rabbit, or guinea-pig.

* Received for publication, March 29, 1943.

Two years later, unfamiliar then with Ruyter's paper, I described the cellular apparatus in the afferent artery of the human kidney, made up of elements of which I gave the following characterization: "At first sight they seem almost spherical or polygonal but on closer examination they seem to emit protoplasmic expansions interlaced with those of the neighboring cells. . . . The nucleus, containing a very delicate chromatin network, is spherical or somewhat indented. The cytoplasm is relatively clear and contains fine acidophilic granulations. These granulations exist constantly." Subsequently, these cells were identified by Goormaghtigh with those studied by Ruyter in mice, and Goormaghtigh himself found granular cells in various animals, especially in the rabbit in which Ruyter had been unable to detect them. The homology of all these cells, convincing as it may appear, remains still to be proved because the cytological aspect of the granular cells in the afferent arteriole is not at all the same in various species and it is quite possible, as we shall indeed see later, that different types of cells have to be distinguished. Nevertheless, Goormaghtigh's study seemed to have clarified the question somewhat until new confusion was created by Zimmermann's article on the so-called "Polkissen" cells in the mammalian kidney. The elements described under this denomination are spherical, or slightly elongated, clear cells occupying the place of the muscular cells in the media and often grouped in multiple layers around the endothelium. At the arteriocapsular junction they often form an eccentric thickening of the vascular wall, giving rise to a structure which Zimmermann compares to a cushion. Zimmermann was not familiar with my publication on the granular cells in the human kidney but he repeatedly referred to Ruyter's paper. Concerning the relationship between his "Polkissen" cells and Ruyter's cells, he made no clear-cut statement. He admitted that Ruyter must have seen the "Polkissen" cells when he described the special structure of the afferent arteriole, but he repeatedly insisted upon the fact that the cells described by him are devoid of granulations. This statement, however, appears considerably weakened when one looks at Figure 5 of his article in which granular cells, absolutely identical with those described by me in the human kidney, are clearly depicted.

All seem to agree that the afferent arteriole, especially in the vicinity of the glomerular junction, offers some structural peculiarities due to the presence of cells described as granular by some and as clear by others. Actually, both types of cells may be present and I shall consider them separately.

Granular cells exist without doubt in many species, man, guinea-pig, cat, dog, rabbit, rat, mouse; but it must be admitted that the aspect

of these cells varies to a considerable degree. For the study of this question the technic is of great importance and the best results are obtained with the method I indicated in my first publication: fixation of absolutely fresh material in Zenker's fluid-formaldehyde solution and Masson's trichrome staining with hematoxylin, fuchsin and aniline blue. With this technic Ruyter's cells stand out clearly and appear to be filled with voluminous, brightly stained red granules. The cells in the human kidney are larger and their granulations, distinctly fuchsinophilic, are much more delicate and scattered. Incidentally, one can understand that Ruyter, accustomed as he must have been to the bright aspect of the granular cells in the mouse kidney, failed to find similar elements in human material. It might have been also that the human kidneys at his disposal were imperfectly fixed or stained. I have often noticed that in kidneys fixed some time after death or in formaldehyde solution, the granulations are absent and the cells appear to have a clear vacuolar cytoplasm. There are thus two types of granular cells, the mouse type and the human type, and the cells in the various species resemble either one or the other.

But there is another very important discrimination which has to be made and which concerns the histochemical reactions of these granulations. In the rabbit especially, but also in other species, granular cells may be observed not only in the afferent arteriole but also in other vessels of the kidney, with distinctly metachromatic granules easily stainable with toluidin blue. These cells are evidently different from those described by Ruyter and by myself. They are mastocytes. This fact, as we shall see, is of great importance in the conception of a secretory activity connected with the granular cells. For the moment, it is interesting to note that mastocytes, which according to Masson are a constant finding in digital glomera, occur also in the renal arteries in contact with glomic structures. The presence of these cells in such delicate and important vascular organs might be connected with their ability to secrete heparin and in this way to prevent thrombus formation.

What, then, is the significance of the other granular cells? In this respect it is interesting to note that both Ruyter and myself referred to these elements as characteristic for the so-called glomic structures in arteriovenous anastomoses, but that our references appeared to be rather inconsistent. Ruyter compared his cells with the "epithelioid" cells described by v. Schumacher in the coccygeal glomus. But v. Schumacher in his studies never mentioned granular cells. He always described clear cells and insisted repeatedly upon the absence of granulations. In my description, I referred to illustrations presented by

Masson (1924) in his article on digital glomera and glomus tumors. Figures 2 and 5 in this article show glomic cells with distinct acidophilic granulations, and it was because of these pictures that I affirmed the identity between the granular cells in the kidney and the neuromuscular elements in the glomic organs. I was surprised at that time that Masson apparently did not pay attention to these granulations in his text. Upon a recent personal inquiry, Masson re-examined his preparations and informed me that in his opinion the elements depicted are not granulations at all, but merely transverse sections of myofibrils. The granulations, however, in the human kidney cells and also those in mouse kidneys are certainly true granulations and not sectioned fibers. But Masson admits that "in some glomic tumors (never in normal glomi) I have seen cells filled with purely acidophilic granulations without any affinity for the basic blues. These cells are certainly modified tumor cells and it is not impossible that they are of the same nature as those which you have observed in the human kidney."

The granular cells which were considered by Ruyter and by myself as characteristic elements of glomic structures appear to be nonexistent in normal arteriovenous anastomoses. They are a special feature of the preglomerular apparatus in the kidney but they may occur in glomus tumors. Their real significance is unknown. Like the clear cells they may be transformed muscle cells. Ruyter has observed all transitional states between muscle cells and granular elements and it is known that under certain circumstances, as in some tumors, muscle cells may assume a typical granular aspect. Murray and Stout, in a recent study, based upon the behavior of glomus tumors in tissue cultures, have identified the so-called epithelioid cells with Zimmermann's pericytes. I have never been able, however, to obtain an impregnation of the granular cells in the kidney with Kopsch's method, used by Zimmermann for the demonstration of pericytes, and it appears to me that as far as the preglomerular apparatus is concerned the identification with pericytes is very doubtful.

Besides the granular cells, the afferent arteriole may contain in its preglomerular portion a certain number of clear cells, as pointed out by Zimmermann. The difference between the two cell types is merely the presence or absence of granulations and if we consider the fact that, in the human kidney at least, the granulations are often few in number and located in a limited region of the cytoplasm, it may be very difficult to state whether a cell belongs to one type or another. In the human kidney, most, if not all, cells of the preglomerular apparatus are granular. Very probably the granular cells are closely related to, if not identical with, the clear cells. Both the granular cells and the

clear cells are connected among themselves by cytoplasmic expansions and they are only imperfectly separated by a loose reticular network. In this respect they show exactly the same behavior as the so-called epithelioid cells in the glomic organs.

This fact must be remembered in order to avoid the confusion which has been created between the epithelioid cells in glomic structures and clear muscle cells. Under certain still undetermined circumstances it happens that smooth muscle cells, especially in vascular walls, take a clear aspect. This is seen especially in arterial walls undergoing hypertrophy, as was recently shown in an eclamptic kidney by Graef. But those muscle cells are distinctly separated by fine collagenous membranes and have no relation to the epithelioid cells in arteriovenous anastomoses.

The whole structural modification which characterizes the pre-glomerular portion of the afferent arteriole—the disappearance of the internal elastic membrane and the replacement of the muscular elements by epithelioid cells—closely resembles the distinctive morphological features of a glomic vessel. Such an identification, however, would be incomplete without a special consideration of the nervous connections. The studies of Stoerk, Dogiel and Masson have shown the extreme development of nervous plexus and sensory end-organs in contact with glomic vessels. Masson, especially, has described the connections of the nerves in digital glomera with the arterial, venous and dermal plexuses and called attention to the close relationship between the so-called epithelioid cells and the richly developed nervous plexus surrounding the adventitia of the glomic vessels. In accordance with his opinion, the nervous elements and especially the Schwann cells are in morphological continuity with the epithelioid cells, so that it is often impossible to draw a definite line of demarcation. Also, these close connections seem to confer on the glomic cells some sort of "neurility" or conductivity, perhaps not unlike that existing in the cells of Hiss' bundle with which, incidentally, they have, owing to their intercommunications and their clear appearance, some morphological resemblance.

It appeared to be of fundamental importance to study the nervous connections of the preglomerular apparatus; but from the beginning I met with considerable technical difficulty. Only impregnations can give reliable pictures of the delicate nervous structures involved and these technics, as everybody knows, are particularly hazardous as far as vascular nerves are concerned. In fact they seem even more difficult in the kidney than elsewhere. I have tried Bielschowsky's method and multiple variations without the slightest success. Cajal's chloral

method, which gave remarkable results in Masson's work on digital glomera, was ineffective in the kidney arterioles. Finally I obtained some successful impregnations by the Gros-Schultze technic* but, here too, hundreds of sections had to be prepared to obtain even a small number of satisfactory impregnations.

Frozen sections of human kidneys fixed in a 4 per cent solution of formaldehyde were washed for 30 minutes in two baths of distilled water. Some drops of nicotine (5 drops to 30 ml. of distilled water) were added to the first bath. The impregnation was carried out in 20 per cent silver nitrate for 10 minutes and then sections were passed for 20 minutes through 5 successive baths of 8 per cent formaldehyde. They were then immersed for 1 to 3 minutes in a 20 per cent Fontana solution, run through ammonia water, acetic water and finally toned for 1 hour in 1 per cent gold chloride. I found it preferable always to employ freshly prepared Fontana's solution. Congested kidneys in general gave unsatisfactory results.

With this technic I obtained in several instances good impregnations of the perivascular nerves which show the existence of a highly developed nervous plexus around the preglomerular portion of the afferent arteriole. This plexus seems to be made up mostly of two branches, one accompanying the afferent arteriole from its origin and another coming in laterally from the interstitial spaces of the surrounding parenchyma. Upon meeting, these branches form a dense network of fibers which gives rise to nerve endings in contact with the cells of the arterial wall. Some of these fibers are coarser and may well be myelinated though I was never able to show myelinated nerves up to the preglomerular portion of the afferent arteriole. The plexus is again split up. Some small fibers appear to penetrate into the glomerulus; others are directed towards the peripheral leaf of the capsule; the main portion turns around the glomerulus and quickly divides in the interstitial tissue of the surrounding parenchyma. Owing to the fact that I was unable to demonstrate nerve endings in the epithelial cells by the Gros-Schultze technic, it is impossible to say whether all of these nerves are purely vascular or whether this plexus is connected with so-called secretory nerves.

There can be no doubt that the preglomerular portion of the afferent arteriole is richly innervated and these results complete the histological picture of a glomic vessel. The special cells located in this portion of the vessel build up a neuromuscular sheath, as I pointed out in my first communication. They constitute a peripheral vasomotor organ,

* Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia, 1938, pp. 227–228.

the localization of which, at the entrance of the glomerulus, is of obvious significance. It is known that the renal glomeruli are intermittently functioning organs. The activity of this vasomotor organ regulates the glomerular circulation in diverting more or less blood from the glomerulus to Isaac-Ludwig's artery* or towards communicating branches which, according to Kosugi, exist between the afferent and efferent arterioles. At all events the preglomerular apparatus seems to fulfill the function common to all glomic organs, which is the alternating exclusion of a certain capillary territory under the close control of the nervous system.

We come now to the second point of our discussion which concerns the relationship between the preglomerular apparatus and hypertension.

Since I first studied this structure my attention has been focused on this problem, and in hundreds of cases I have tried to compare the development of the preglomerular apparatus in kidneys of normal and hypertensive patients. I soon realized the difficulties and the unavoidable shortcomings of such a comparative study. The preglomerular apparatus is very irregularly developed in the various afferent arterioles. Moreover, the cells are generally accumulated at one side of the vessel so that for each glomerulus there is only one plane of sectioning which gives the complete picture of the apparatus. In an average kidney section, containing about one hundred glomeruli, there are only five or ten which give a fairly accurate impression of these vascular structures. Even if these one hundred glomeruli are examined in serial sections, this gives only a 1 to 10,000 approximation of the situation in the whole kidney. Obviously this is insufficient to make any precise statement about the development of the preglomerular apparatus and to establish comparative figures.

Furthermore, there are still many imponderable factors involved. The same fixation does not always give the same results. It so happens that sometimes the cells are poorly fixed, the granulations are not visible, the cytoplasm is uniformly clear and vacuolar and often the cell boundaries disappear so that the whole structure becomes indiscernible to even an experienced observer. Comparative studies become more or less a question of personal impression and the results may show surprising variations if, as I have done repeatedly, whole series of cases are submitted to different observers.

The results of these studies carried out in France as well as in this country may be summarized as follows. The preglomerular apparatus shows appreciable variation in its development in various persons but

* Smith, H. W. Physiology of the renal circulation. *Harvey Lectures*, 1940, **35**, 166–222.

there is no parallelism whatsoever between the degree of development and hypertension. Considerably developed structures with abundant granular cells may be seen in children and adults without any elevation of the blood pressure.

In cases of hypertension with hyaline degeneration of the afferent arteriole, the preglomerular apparatus is often well preserved. Sometimes one even has the impression of a certain degree of hyperplasia. But when the arteriolar lesions are more pronounced the apparatus is almost always affected. Fat infiltration of the cells is often present. The granulations disappear and the nuclei become pyknotic. Finally the cells disappear. In the malignant forms of hypertension I always found the cells degenerated or completely destroyed.

These findings are in opposition to those of Elaut and Goormaghtigh. Elaut, in 1934, reported hypertrophy of the epithelioid cells in dogs rendered hypertensive by denervation of the aortic arch and the carotid sinus. Goormaghtigh (1939) then carried out research along the same line and confirmed Elaut's findings, but in subsequent studies laid more and more emphasis upon the glandular nature of the granular cells. In dogs and rabbits made hypertensive by the Goldblatt-Grimson technic* he noticed a considerable hyperplasia and multiplication of the granular cells which appear all over the arterial system in the kidney from the hilum to the glomerular tufts. The cells proliferate so abundantly that their accumulation causes mechanical circulatory troubles. Furthermore, they show not only acidophilic but basophilic granulations and fat droplets, suggesting a real secretory cycle as distinct in Goormaghtigh's opinion as the one observed in the glandular cells of the pituitary body. Owing to the fact that the stimulation of these cells appears to be the distinctive feature of hypertension, Goormaghtigh concluded that these granular cells form an endocrine gland scattered through the kidney like the Langerhans' islands in the pancreas and that their main function is the secretion of the hormone-like pressor substance, renin.

The main objection I can make with regard to these conclusions is that in human kidneys I have never observed pictures like those described in dogs and rabbits. Furthermore, if renin is responsible for hypertension it is surely not secreted by the granular cells of the preglomerular apparatus owing to the fact that in the severest cases of hypertension these cells are generally degenerated. But there are other possibilities which might well explain Goormaghtigh's observations. When we read that the granular cells which proliferate in those kidneys

* Grimson, K. S. The onset of renal ischaemia hypertension induced by readily adjustable renal artery clamps. *J. Physiol.*, 1939, 95, 45–P to 46–P.

contain granulations of all kinds and especially basophilic ones; and that they form, by their accumulation, bulging pads in the intima which secondarily are infiltrated by fats, degenerate and form sclerotic plaques, then it becomes almost evident that these cells are mastocytes and histiocytes. The whole process described therefore has nothing to do with the preglomerular apparatus. It is merely one of muscular hypertrophy and of arteriosclerosis of the renal vessels and it becomes difficult to see here the activity of endocrine elements responsible for the increase of the blood pressure. These lesions are probably a result of hypertension and not its cause.

In rejecting Goormaghtigh's interpretations I am far from ruling out the intervention of the preglomerular apparatus in the mechanism of hypertension, but in my opinion the available data point in a direction just opposite to that indicated by Goormaghtigh. The regressive changes which the preglomerular apparatus undergoes constantly in advanced and severe cases of hypertension indicate the suppression of a delicate regulatory mechanism placed at the entrance of the glomerular circulatory system. It is well known that at a certain moment hypertension, which may be, for long years, a reversible phenomenon, becomes stabilized at a high level and it is not impossible that the destruction of the preglomerular apparatus is connected with this change which plays such an important rôle in the evolution of the disease.

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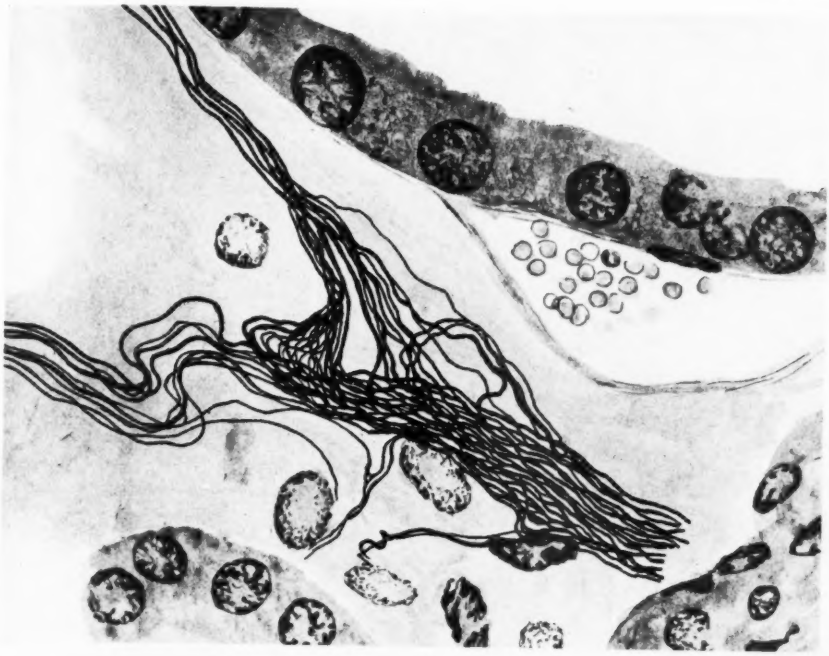
DESCRIPTION OF PLATES

PLATE 28

FIG. 1. Nervous plexus of the afferent arteriole near the capsular junction. The lower left branch accompanies the afferent arteriole. Various nerves arise from the plexus and one nerve ending is seen in contact with a cell of the preglomerular apparatus. × 1100.

FIG. 2. Photomicrograph of the same plexus. × 1225.

1



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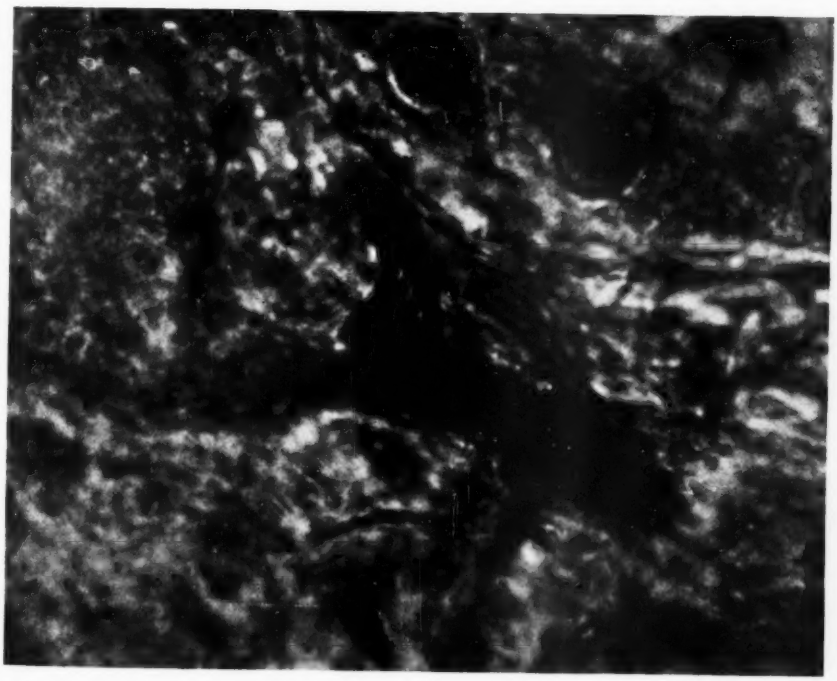


Oberling

Preglomerular Cellular Apparatus

PLATE 29

FIG. 3. Another view of a preglomerular nervous plexus. The lateral branch is here much smaller than in Figure 2. × 745.

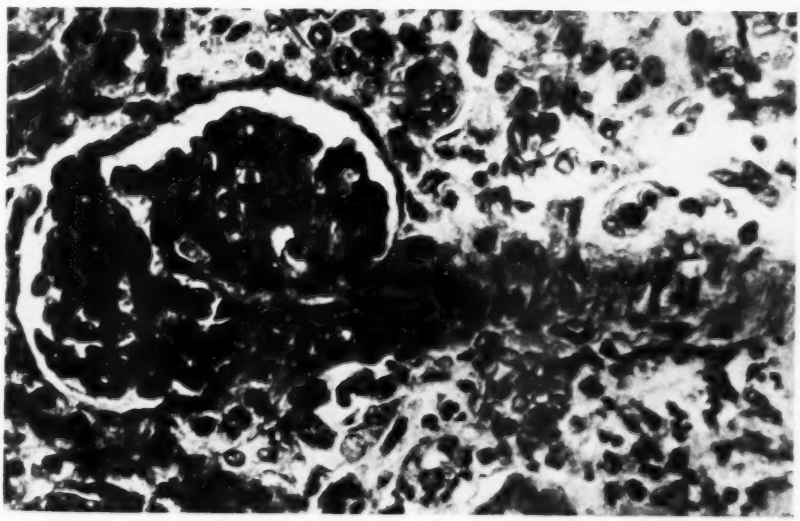
FIG. 4. Preglomerular apparatus in a kidney of a child, 8 weeks old. The afferent arteriole runs in a straight line towards the glomerulus. Near the capsular junction the vessel shows the typical modification of its wall characterized by the presence of large cells. × 330.

FIG. 5. Preglomerular apparatus in the same kidney. At this magnification the granular cells surrounding the afferent arteriole appear very distinctly. × 830.

3

5

4



Oberling

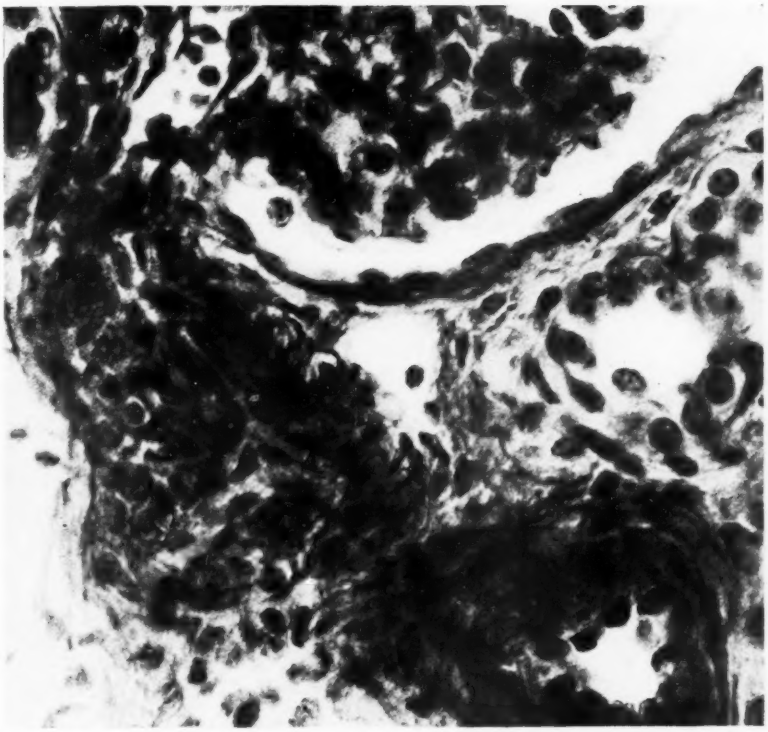
Preglomerular Cellular Apparatus

PLATE 30

FIG. 6. Normal kidney of a 24-year-old male. Glomus-like structure around the bifurcation of the afferent arteriole and Isaac-Ludwig's arteriole. The latter is seen branching to the left. × 570.

FIG. 7. White male, 50 years of age; hypertension for many years; died of apoplexy. The photomicrograph shows the afferent arteriole and Isaac-Ludwig's arteriole with hyaline degeneration of their walls. The preglomerular apparatus located near the capsular junction in contact with the macula densa shows retracted and vacuolar cells with pyknotic nuclei. Frozen sections from this case, stained with scarlet red, show marked fat infiltration of the preglomerular cells. × 570.

6



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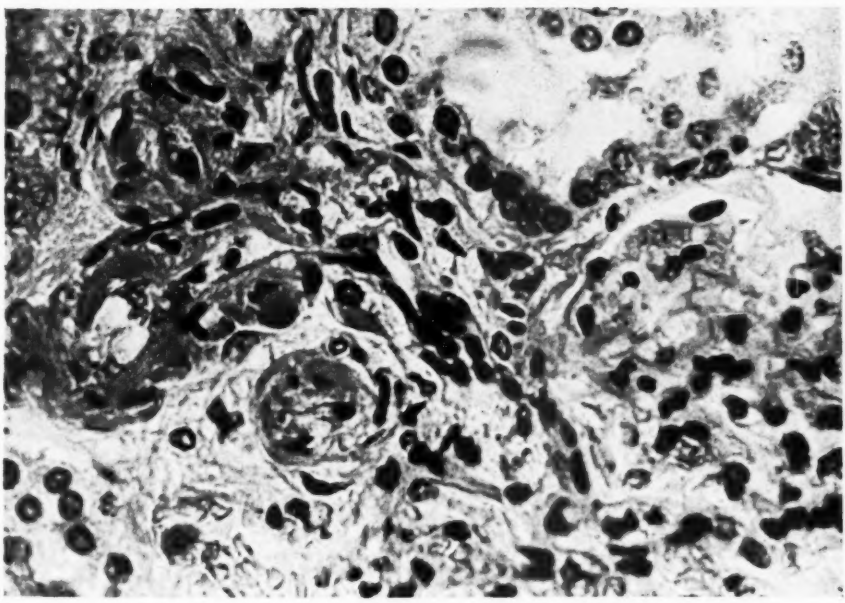
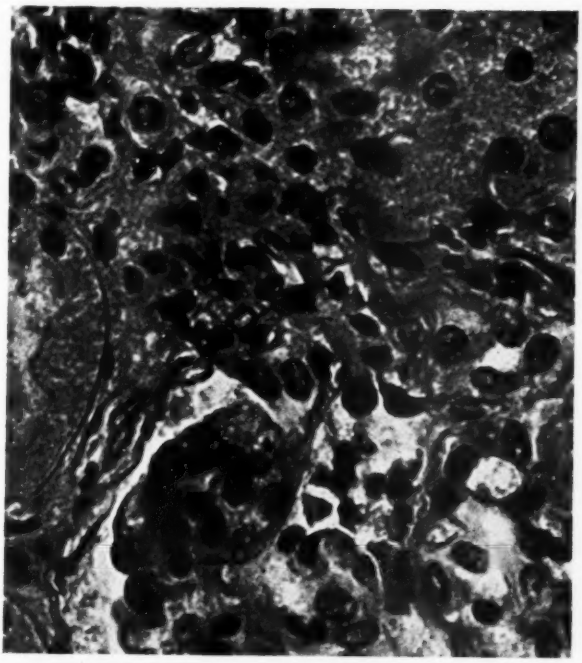


Oberling Preglomerular Cellular Apparatus

PLATE 31

FIGS. 8 and 9. White female, 65 years old; obesity; hypertension: died of cardiac insufficiency. Pronounced degenerative lesions of all the preglomerular cells which present pyknotic nuclei and a homogeneous or vacuolar cytoplasm. × 600.

8



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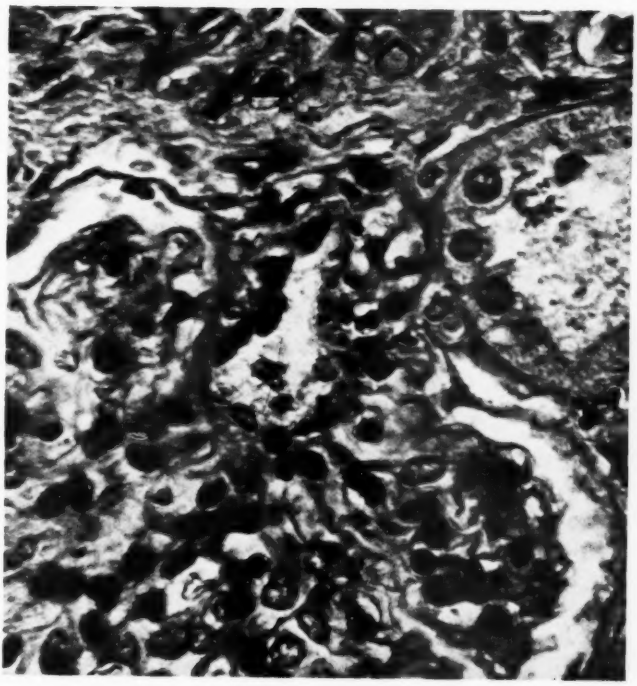

Oberling Preglomerular Cellular Apparatus

GENERALIZED VACCINIA WITH DUAL VIRUS INFECTION
A Case Report *

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Dual or multiple virus infections have been demonstrated experimentally[1-3] by inoculation of known viruses in appropriate animals. Different varieties of specific inclusion bodies have been seen[3] in an individual host cell. Certain virus infections appear to maintain their usual characteristics in the presence of another virus while other dual or mixed virus infections exert an interference phenomenon.[4]

This preliminary report concerns the demonstration of both cytoplasmic and intranuclear inclusions in material obtained from a clinical case of generalized vaccinia.

Report of Case

J. R. (B.C.H. no. 100152), a white male infant, 1 year of age, the youngest of three siblings, was admitted to the Boston City Hospital on July 6, 1941, with a generalized skin eruption and high fever.

Ten days before admission the patient and a 4-year-old sister were vaccinated by the epidermal scratch method. The sister followed the ordinary course of vaccinia. Six days after vaccination, the patient had a temperature of 104°F. and a vesicle appeared at the site of vaccination. Within a few hours, other vesicles appeared around the vaccination site. The following day, vesicles were observed over the entire left arm and 2 days later, or 9 days after the vaccination, numerous vesicles were present over the legs, arms, face and axillae. A persistent cough developed simultaneously with the first appearance of the vesicles.

The family history was not contributory.

The patient had been bottle fed since birth. His development and general health were considered good. At the age of 2 months he had had pertussis which was followed by two bouts of bronchopneumonia, the second attack being 8 months prior to the present illness. Recovery was complete. Recurrent eczema had been present since the age of 3 months but none was present at the time of vaccination. He was found to be sensitive to string beans, tomatoes, spinach, eggs, oranges, and wheat.

Physical examination revealed a critically ill, dehydrated, well developed and well nourished, mentally alert male infant, with a temperature of 104°F., a pulse rate of 152 beats per minute and a respiration rate of 50 per minute. Breathing was rapid and grunting. Over both arms were many discrete and confluent, round, shotty, umbilicated pustules. Similar semiconfluent umbilicated pustules were present on the legs in great numbers, and a few were scattered over the skin of the face, neck, axillae, anterior chest and abdomen. All lesions were fundamentally of identical appearance. No lesions were present on the palms, soles, or oral mucosa. There was moderate enlargement of the cervical and right inguinal lymph nodes. The nodes of the left axilla were markedly increased in size. There was slightly impaired resonance over the left upper chest with coarse bronchovesicular breathing and rare

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† Now in the Medical Corps, A.U.S.

moist râles. Edema of a moderate degree was present in both arms. There were no other positive physical signs.

The urine was essentially negative. The blood cell counts were within normal limits and the Hinton test on the blood was negative. Hemolytic *Staphylococcus aureus* was cultured from the pustules.

Course. Fluids were given by mouth. Sulfathiazole, grains 7½ (0.5 gm.), was given every 4 hours for 6 days. The skin lesions were treated with a 1:10 solution of chlorated soda, and powdered zinc stearate.

The temperature rose to 106.2°F. (rectal) 2 days after admission and a generalized convulsion ensued. Cold sponge baths reduced the temperature and the patient improved. Several convulsions occurred during the course of the illness, when the temperature became 104°F. or higher. Occasionally the patient became cyanotic, necessitating oxygen therapy. The signs of bronchopneumonia rapidly disappeared. The pustular lesions crusted slowly, finally healing with only slight residual scarring. The patient was discharged entirely well 38 days after admission to the hospital.

Experimental Procedures

Scrapings from the pustular skin lesions 12 days after the first appearance of the generalized skin eruption were inoculated on the corneas of a fully grown female and a male rabbit. Each testicle of the male rabbit was inoculated with 0.1 cc. of a saline suspension of the initial scrapings. The female rabbit was sacrificed after 96 hours, the male after 124 hours. Exudate and small, raised areas suggestive of vesicles appeared over the corneas and conjunctivae of both animals in 72 hours, reaching a maximum in 96 hours. The testicles became firm in 72 hours, reaching a maximum induration in 96 hours. The rectal temperatures of the rabbits varied from 105° F. at 72 hours to 103° F. in 96 hours. The temperature of the male rabbit returned to normal within 120 hours.

Portions of each eye and testicle were removed from the sacrificed animals immediately after death and fixed in Zenker's solution. Histological sections were stained with the phloxine and methylene blue stain. Portions of each eye and testicle were also saved fresh for transfer.

Microscopically, the corneal lesions were consistent with lesions produced by vaccinia alone. There was marked ulceration of the cornea with an acute inflammatory reaction consisting of polymorphonuclear leukocytes, macrophages and strands of fibrin. About the periphery of the ulceration the majority of the epithelial cells contained cytoplasmic inclusions which were typical of Guarnieri's bodies of vaccinia.

In addition to the cytoplasmic inclusions, intranuclear inclusions were present. These were few in any one lesion of the cornea or testicle. Stained with phloxine and methylene blue, they appeared as homogeneous, brilliant pink masses pushing the nuclear chromatin toward the nuclear membranes, producing a margination of the nuclear

chromatin about the masses. The intranuclear and cytoplasmic inclusions, as far as could be determined, were present only in epithelial cells. In rare instances intranuclear and cytoplasmic inclusions were present in the same cell.

Part of the fresh cornea of the female rabbit originally inoculated was finely ground and suspended in physiological saline solution and inoculated on the cornea of a second anesthetized rabbit and so on through thirty-nine consecutive transfers. It was thought best not to use testicular material or continue testicular inoculation for fear of introducing Virus III into an already complicated picture.

The intranuclear inclusions continued to appear in approximately the same numbers as in the original inoculation. The cytoplasmic inclusions were usually numerous.

Since the inclusions of vaccinia are only cytoplasmic, it follows that either two viruses were responsible for the lesions or that one virus producing both cytoplasmic and intranuclear inclusions was being transmitted. Variola characteristically produces both cytoplasmic and intranuclear inclusions in man but it has been shown [5] that material obtained from variola lesions and inoculated on a rabbit's cornea produces lesions identical with vaccinia; that is, the specific cytoplasmic inclusions of vaccinia.

Paravaccinia is also characterized by cytoplasmic and intranuclear inclusions but can be excluded, as man is the only known susceptible host.[6]

On the assumption that I was dealing with a dual virus infection, attempts were made to separate the virus producing intranuclear inclusions from that causing the cytoplasmic inclusion. Five rabbits were immunized with a stock vaccine virus. Nineteen days later one was inoculated on the cornea with a suspension of the cornea from the early rabbit passage and subsequent consecutive transfers were carried through the immunized rabbits. The cytoplasmic inclusions of vaccinia as well as the unknown intranuclear inclusions failed to appear. It was impossible to demonstrate intranuclear inclusions in the absence of the cytoplasmic inclusions in any of the rabbits immunized. Control or nonimmunized rabbits inoculated with the same suspension showed both the cytoplasmic inclusions of vaccinia and the unidentified intranuclear inclusions.

The possibility that the intranuclear inclusions might be those of herpes was investigated. Three rabbits were inoculated intracerebrally with a saline suspension of finely ground corneal lesions, but these failed to show any characteristic of herpes encephalitis nor were lesions demonstrable histologically. It is interesting to note that recently

Anderson[3] inoculated herpes simplex and vaccinia virus on the chorioallantois of chick embryos, producing dual infections. Doubly infected cells occurred but rarely and only in areas of the epithelium where vaccinial infection merged into foci which were purely herpetic.

The viruses of varicella and herpes zoster are characterized by producing only intranuclear inclusions. Both can be excluded as only possibilities for as yet it remains to be demonstrated conclusively that either can be transmitted to animals.

The question arises whether Virus III might have been present in the two rabbits originally inoculated and have been repeatedly transferred through thirty-nine transfers. If such were the case it is to be expected that by repeated transfers the Virus III would become more prominent and readily transferred to rabbits immune to vaccinia, but this did not occur in our experiments.

The chorioallantois of 12- to 14-day-old chick embryos was employed in an attempt to accentuate the growth of the intranuclear inclusions. However, the cytoplasmic inclusions so overshadowed the intranuclear inclusions as to make their identification extremely difficult. Emulsions of previously inoculated chorioallantoic membranes were inoculated on the corneas of rabbits but with no significant increase in the number of intranuclear inclusions over rabbit cornea-to-cornea transfers.

Guinea-pigs were employed as experimental hosts through seven consecutive transfers. The virus was inoculated on the cornea. In no case were intranuclear inclusions found, while cytoplasmic inclusions were numerous.

Six white mice were inoculated intraperitoneally and intracerebrally with saline suspensions of rabbit corneal lesions. No untoward reactions resulted and histological examination failed to reveal inclusions of any sort in the brains or in the viscera.

The conclusion therefore was reached that the intranuclear inclusions may represent a new virus or a known virus so altered by the symbiotic presence or interference phenomenon of vaccinia as to render it difficult to identify. It is possible that the original vaccine given the patient was contaminated by another virus. Unfortunately, it was impossible to study the original vaccine. It is also possible that the patient was harboring a virus which was activated by the vaccination. It is recognized that generalized vaccinia is most apt to occur in persons susceptible to eczema or other dermatological disorders. Since I did not have the patient's skin lesions available for biopsy, the possibility of pre-existing virus infection cannot be determined.

SUMMARY

Cytoplasmic and intranuclear inclusions were demonstrated by inoculation of material from a case of generalized vaccinia into animals. The cytoplasmic inclusions were identified as vaccinia but the intranuclear inclusions were not identified. The intranuclear inclusions failed to appear in the absence of vaccinia although in combination the two viruses were readily transmitted through thirty-nine consecutive rabbit transfers. The virus producing the intranuclear inclusions did not behave in experimental animals in a manner characteristic of known viruses producing intranuclear inclusions.

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OBSERVATIONS ON THE STRUCTURE OF BONE IN ESTROGEN-TREATED COCKS AND DRAKES *

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Among the many hormones which influence the finer structure of bone, estrogens have, during the past several years, attracted particular attention, and it is now well established that experimental treatment may lead to profound modifications in the internal configuration of bones. Rats,[1-8] mice,[9-14] guinea-pigs,[3-5, 13, 15, 16] chickens,[8, 17] ducks,[18] pigeons [19-21] and sparrows, [22, 23] all show these effects, although the amount of response varies from one species to another and also with sex, age and other intrinsic factors.

A number of investigators have studied the histologic consequences of estrogen treatment on bone, especially on the long bones of young and growing mammals. The present report is concerned with observations on long bones of estrogen-treated cocks and drakes, and particularly with estrogen-mediated changes in the bones of mature birds.

Information concerning the animals which were used in this work and the doses of hormone which were injected is contained in previous publications.[8, 17, 18] Briefly, the work of one of us (B. Z.) was done with White Leghorn cockerels which were 6 weeks of age when treatment was started, while the experimental material of the other (W. L.) consisted of mature White Leghorn and Creeper cocks and of mature Pekin and Mallard drakes. The hormone used was estradiol benzoate. For details concerning duration of treatment and dosage we refer to our earlier publications.

ROENTGENOGRAPHIC OBSERVATIONS

Young Cockerels

X-ray pictures of the femur and tibia were taken, at the time of autopsy, of those cockerels which had been treated with estrogen after reaching the age of 6 weeks. Drastic changes occurred in some of these animals. After prolonged treatment the long bones failed to attain their normal length, with a tendency for the tibia to be relatively shorter than the femur. The earliest roentgenographic signs of bone changes were visible in a cockerel which had, during 21 days, received injections of 5 mg. of estradiol benzoate three times a week. There was a diffuse increase in density in the shaft and in the epiphyseal ends of the femur

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and, to a lesser degree, in the tibia. In the metaphyseal regions the changes were less uniform; there were scattered islands of particularly dense bone. In another cockerel treated similarly, but for 5 weeks, bone density was still further increased (Fig. 1). In yet other cases, treated for longer periods, the bones were very irregular in density. There were indications of the removal of bone salts from the epiphyseal region and from the shaft, but there were also, scattered throughout the shaft, islands of very dense bone (Fig. 2). In extreme cases, after long-continued treatment, only traces of the marrow cavity remained in the femur (Fig. 3).

Of the estrogen-treated adult cocks, roentgenograms were taken of only the humerus, ulna, radius and tarsometatarsus. No modifications due to treatment could be seen in these bones.

Ducks

In the humerus of adult Mallard drakes no response to estrogen treatment was visible in roentgenograms. In mature Pekin drakes, on the other hand, a slight and diffuse increase of density was noticeable in the distal half of the shaft. This was most pronounced in the animal which had received the largest amount of estrogen.

As in cocks, the femora of drakes responded more to estrogen administration than any other long bone. In Mallard drakes the roentgenograms indicated but slight changes in the structure of the femur. In the most advanced cases (highest dose of estrogen) there was a diffuse increase of bone density, especially in the central section of the shaft and near the joint surfaces. In contrast to this, the femora of Pekin drakes had undergone extreme changes under the influence of estrogen administration, and these changes showed a definite relationship to the dosage of hormone which was employed (Fig. 4). Density of the bone shadows was much increased and in the most extreme case (drake P_5) the marrow cavity appeared nearly obliterated. Formation of new bone was greatest at the epiphyseal ends, where normally the number of endosteal trabeculae is largest, and, vice versa, the least increase in bone deposition occurred where the fewest trabeculae are normally present, *viz.*, in the middle part of the diaphysis.

No radiographic changes could be seen in the tibia of Mallard drakes; but, in the Pekin drakes the modifications of tibial structure were conspicuous. With the lowest dosage of estrogen (drake P_1) bone density was increased chiefly in the region of the proximal epiphysis and metaphysis and to a lesser degree along the inner surface of the shaft. In addition to these modifications there were, after greater hormone dosage, many small localized areas of increased density throughout the

upper half of the tibial diaphysis. The lower part of the tibial shaft and the distal epiphyseal region appeared nearly or entirely free of changes (Fig. 5).

No radiographic signs of estrogen effects could be seen in the tarsometatarsal bones of either Mallard or Pekin drakes.

Gross Observations

The following observations relate to long bones, of mature experimental and control animals, after they had been sawed in half frontally, cleaned, bleached and defatted.

Cocks

After relatively low doses of estrogen (total of 1.5 to 2 mg. in 27 days) the long leg bones of four adult cocks showed no macroscopic changes. In another group of four cocks which had been injected with 7 to 9 mg. of estrogen in the course of 2 months the responses were definite. In the femur the inner surface of the shaft and all endosteal trabeculae were covered by a thin layer of new bone. The same, though to a lesser extent, was true for the tibia, except that the distal metaphysis and epiphysis were free of new bone deposits. The tarsometatarsus showed no reaction at any dosage level. In two capons which were treated with somewhat larger doses (11.5 and 12.5 mg. of estrogen, respectively, during 2 months) the changes were about the same as those in the last group, except that there was a more uniform response throughout the whole length of the tibia. The results again were similar after still greater amounts of estrogen, administered during shorter or longer intervals. Within certain limits the osseous changes increased in extent *pari passu* with the amount of estrogen which had been given. The imprint of high doses of estrogen on bone structure may, however, be less extensive than that of smaller doses. This is illustrated by the following examples. In a group of four cocks which received 2 mg. of estrogen daily for 12 and 19 days, respectively, the two which had been treated for 19 days showed definitely less new bone than the animals which had been injected for only 12 days. Again, in a group of three cocks, one received 31 mg. of estrogen in the course of 130 days and another cock was injected with only 23 mg. during the same period. The changes were less pronounced in the former animal than in the latter. Both of these cocks showed less new bone than the third one which was given 7 mg. during 20 days. There is evidence indicating that these results are due to secondary resorption of newly-formed bone.

Ducks

Gross inspection of the long bones of estrogen-treated mature Mallard drakes showed that the changes in the interior structure of bone are less pronounced than in cocks. No indications of response could be seen in the humerus. As far as the long bones of the leg are concerned, the femur reacted most intensely, the tibia less, the tarsometatarsus little, if at all. Within the femur, about equal amounts of new bone were formed in the proximal and distal ends, but much less in the middle part of the diaphysis. It should be emphasized, however, that there was some measure of response in the central section of the shaft. Since this region is, normally, entirely free of trabecular bone, it is clear that estrogen-induced bone formation can occur in the absence of pre-existing endosteal trabeculae. The proximal end of the tibia of Mallards undergoes drastic changes under the influence of estrogen; the effect becomes less conspicuous in the upper half of the shaft and is lacking entirely in the lower third of the diaphysis and in the distal head of the bone.

In Pekin drakes the osseous responses to estrogen were much more extreme than in either Mallards or cocks. There were no grossly visible changes of the humerus. The leg bones showed the same proximo-distal response gradient as that observed in Mallards and cocks, *viz.*, femur > tibia > tarsometatarsus. This sequence holds at all dosage levels. Complete obliteration of the marrow cavity of the femur occurred in extreme cases. The distal end of the femur tended to undergo somewhat more extreme hyperossification than the proximal one, and the central parts of the diaphysis showed gross reactions only to high doses of hormone. In the tibia the proximal end was the most responsive region of the bone; the upper end of the diaphysis followed next; in the lower part of the shaft hyperossification was slight, and was absent in the distal head. There were no grossly observable changes of the tarsometatarsus even at the maximum amounts of estrogen used in our experiments.

Under the influence of the lowest doses of estrogen some trabeculae disappeared from the central part of the diaphyseal shafts of femur and tibia, while simultaneously definite hyperossification took place in other parts of the same bones. Evidence of halisteresis was found also in the tarsometatarsus in which there was no evidence of hyperossification elsewhere.

A comparison of bones from untreated mature animals revealed that femora and tibiae of Mallard drakes have fewer endosteal trabeculae than the same bones of cocks. The reverse, *viz.*, a much greater amount of spongiosa, was found in femora and tibiae of normal Pekin drakes

than in the corresponding bones of either Mallards or cocks. This sequence coincides with that of the degree of response after estrogen treatment, *viz.*, Mallards < cocks < Pekins. A similar relationship exists with regard to different parts of one and the same bone; for instance, there is less (and coarser) spongiosa in the central section of the femoral diaphysis than in other parts and this region is the least responsive.

In a general way, the degree of hyperossification of femur and tibia was the greater the higher had been the amounts of injected estrogen (duration of treatment being the same). In the Mallard and Pekin drakes the results from the highest dosage level of our tests, 1 mg. daily for 3 weeks, appeared to approach the maximum response obtainable; for the effect of 1 mg. daily was not much greater, with regard to degree of hyperossification, than that of 0.5 mg. per day.

Histologic Observations *

Cocks

Following the lowest dose of estrogen which we gave to adult cocks (nos. 5646 and 3973, 0.17 to 0.23 mg. of estradiol benzoate daily for 4 weeks) the outstanding feature of the long bones was destruction and resorption of bone. This was most marked in the femur (Fig. 6), less so in the tibia, and least in the tarsometatarsus. There was slight thinning of the femoral and tibial shafts; the periosteal bone showed splotchy staining; many haversian canals had an increased diameter and their number was greater than normal; the borders of the haversian canals frequently had a frayed appearance and accumulations of detritus could be seen; there were Volkmann's canals and cavities containing osteoclasts. Vascularization was increased. In some regions, especially in the distal end of the femur, the processes of bone reconstruction extended to the endosteal trabeculae. In the marrow cavity the lymphocytes were often replaced, to a greater or lesser extent, by pseudoeosinophils; in other places the reverse was true. There was some evidence of fibrosis of the marrow but this did not assume extensive proportions. In a few places the inner surface of the shaft and the trabeculae which project into the marrow cavity were covered with uncalcified osteoid tissue and there were a few newly-formed endosteal spicules. The number of osteoblasts was increased on the surface of the old and of the more recently formed bone. On the whole, however, there was more evidence of bone destruction or resorption than of formation of new bone.

* The bones were fixed in formaldehyde. For staining we used chiefly Dominici's orange-eosin-toluidin blue method (Romeis, B. Taschenbuch der mikroskopischen Technik. R. Oldenbourg, Munich & Berlin, 1924, ed. 11, p. 140).

As one progresses to animals which had received higher doses of estrogen the signs of bone destruction become less prominent, whereas those of new bone formation increase in conspicuousness. Heightened vascularization was always evident. Fibrous degeneration of marrow, disintegration of marrow cells and accumulations of cell fragments (*i.e.*, focal necrosis) could be seen everywhere. The fibrous degeneration seemed to involve the pseudo-eosinophils more than other cell types. There was evidence of the transformation of small lymphocytes into osteoblast-like cells and finally into bone cells. Considerable amounts of osteoid tissue, in various stages of calcification, could be seen. New endosteal trabeculae appeared along the surface of the marrow cavity and also between old bone spicules. Marrow degeneration and new bone were also found in osseous cavities of the shaft itself (Fig. 7).

In the two cocks which had received the highest doses of estrogen (nos. 97 and 99, 2 mg. of estradiol benzoate daily for 19 days) the extent of the changes in the femur and tibia resembled that in Pekin drakes which had received about half that amount (to be discussed later in this paper). There were many new endosteal trabeculae in the shafts of both bones, but especially in that of the femur. The marrow showed extreme fibrous degeneration and much cell-free granular débris near the newly calcified bone spicules. Vascularization was greatly increased and there were definite signs of thickening of the walls of small blood vessels and of stasis. There were also accumulations of plasma (containing some red cells) outside of the vessels.

Fibrous degeneration of marrow and small amounts of new bone were also observed in the mural osseous cavities of the tarsometatarsal shaft, but not in the marrow cavity of this bone (Fig. 8). The haversian canals of the tarsometatarsus were much enlarged in diameter.

As in the material from ducks, the pseudo-eosinophils were particularly subject to fibrous replacement, whereas the lymphocytes participated to a much less degree in this process. There were large numbers of small lymphocytes in the center of the marrow cavity and some of these were in transition to water-clear osteoblast-like cells which were obviously destined to become bone cells. There was no osteoclastic activity in the bones with most extreme changes.

In young cockerels treated with estrogen the types of reactions and the sequences of events were very similar to those described for mature animals. The extent of response, however, was much greater, and, in extreme cases, led to nearly complete obliteration of the marrow cavity (femur) by newly-formed bone (Fig. 9).

As in the material already described, the first response of the long

bones to estrogen appeared to be increased vascularization, followed by widening of the haversian canals in the shaft, disappearance of fat from the marrow cavity, multiplication of marrow cells, fibrous degeneration of marrow, circulatory stasis and extravasations, production of hyalin from erythroblasts and red cells of the marrow, and finally the formation of new bone (Figs. 10 and 11). All these changes proceeded more rapidly in the femur than in the tibia.

Control animals injected with cholesterol showed increased vascularization and some signs of circulatory stasis and hyaline formation, but no other changes. Control animals which had received injections of olive oil had an increased blood supply to the marrow cavity, but there were no other bone abnormalities.

Pekin Drakes

The bones of estrogen-treated Pekin drakes showed two outstanding features, *viz.*, marrow fibrosis and formation of new bone trabeculae (Fig. 12). These and other processes varied in extent according to the particular region and according to the amount of estrogen which had been injected.

In the drake which had received the lowest dose of estrogen (P_1) there was great distention of the capillaries and blood spaces. All marrow cells were much increased in number, and this increase followed the pattern of normal distribution, *i.e.*, in any particular bone region the normal proportions of different cell types seemed to be more or less preserved. Thus, it was observed that in the proximal tibia of control animals pseudo-eosinophils were found almost exclusively near the endosteal surface of the metaphysis, whereas myelocytes became more and more frequent toward the center of the bone. On the other hand, the predominance of myelocytes in the center of the joint end (in control animals) gradually disappeared toward the shaft, and in the middle region of the diaphysis few cells other than pseudo-eosinophils were present. This order of distribution of different cell types was not affected by the multiplication of cells occurring under the influence of estrogen. Fatty marrow, common in the controls, was much reduced or absent in the treated animals.

Another relationship between normal structure and degree of response to estrogen treatment could be observed in cross sections of the tibial shaft. In normal ducks and domestic fowls the large osseous cavities (Resorptionshöhlen or Bauplätze of Petersen [24]) are restricted to the fibular side of the tibial shaft. Red marrow is formed within the osseous cavities and also along the fibular surface of the marrow cavity, whereas the remainder of the marrow cavity, in adult birds, contains fatty marrow. This structural asymmetry was reflected in the changes

caused by estrogen treatment. Within the osseous cavities of the shaft fibrosis of the marrow was more extreme than elsewhere, and in the marrow cavity more new bone was formed in the fibular part than in the remainder (Fig. 14). If much new bone was present, the higher rate of bone formation on the fibular side of the tibia became especially noticeable by a column of spongiosa bulging, on this side, into the marrow cavity. Again, the occurrence of abnormal arterioles was much more common on the fibular side of the tibia than in other parts.

In the bones of treated animals there was evidence of fibrotic replacement of myelocytes and of pseudo-eosinophils. This marrow fibrosis was the more marked the higher had been the amount of injected estrogen. In extreme cases very little normal marrow appeared to remain (Fig. 15). In some places one could see relatively large amounts of cell-free substance, thus produced. Elsewhere these accumulations were interspersed with smaller or larger numbers of osteoblast-like cells.

On the bony surface of the marrow cavity and along the surface of endosteal trabeculae newly-formed bone was present. The amount of new bone varied with the amount of estrogen which the particular animal had received. In the more extreme cases many trabeculae consisting entirely of new bone were present. They were usually composed of irregular, concentric layers of new bone structure.

These new bone trabeculae were generally, but not invariably, covered with a thin layer of degenerated marrow containing osteoblast-like cells. Fibrous marrow degeneration was especially evident in the vicinity of bone trabeculae. Various stages of calcification of fibrous marrow could be seen.

Multinucleated giant cells (osteoclasts) were numerous along the surface of some of the new bone trabeculae, but only a few were found near others (Fig. 16).

A feature observed only in the animals which had received the largest amounts of estrogen was the occurrence of cartilage within the old endosteal bone (Fig. 17). Some bone trabeculae showed definite signs of halisteresis in the central parts. This seemed to be followed by the appearance of small areas of cartilage. In the more extreme cases only a narrow border of the old bone remained on the surface of a trabecula, the interior having been transformed entirely into large-celled cartilage. All the cartilage cells were oriented at right angles to the long axis of the trabeculae. Another peculiarity which was restricted to birds with the highest dosage levels was the presence of "cysts" in the marrow cavity. These "cysts" had a lining consisting of one layer of cells and presumably represented sections through greatly dilated venules. They were filled with large masses of erythrocytes, with eryth-

rocytes and plasma, or almost exclusively with plasma and but a few red cells. Further signs of stasis were found in many capillaries and venules (Fig. 19). Accumulations of plasma occurred frequently (Figs. 13 and 18) and the arteriolar walls often showed definite hypertrophy of the elastic elements of the media and a relative reduction in the width of the vessel lumen. An increase in vascularization and widening of the capillary lumina was observed in the bone marrow of those animals which had received the lower estrogen doses. This increase in blood supply appeared to be secondary to degenerative changes of the marrow; for cross sections of the diaphysis (femur, tibia), after low and medium doses of estrogen, demonstrated the presence of all structural alterations (marrow fibrosis, increased vascularity, new trabecular bone) in the peripheral parts of the original marrow cavity, whereas the only changes which were noticeable in the center relate to the large lymphocytes which had indistinct nuclei and a slightly acidophilic instead of a basophilic staining quality of the cytoplasm. On the other hand, in animals which had received high doses of estrogen the changes which, on the lower hormone levels were confined to the periphery, extended to the center of the marrow cavity. Accumulations of plasma were also frequently observed outside of vessels.

No indication of new bone formation was found in the humerus of estrogen-treated animals, but there was destruction of old bone throughout the humeral shaft (Fig. 20). There were new Volkmann's canals and alongside them one could observe accumulations of irregular-sized granules of broken-down bone. Disintegration and resorption of bone (osteolysis) could, however, be seen throughout the shaft, independent of the presence of haversian or Volkmann's canals. There were no osteoclasts. Vascularization was increased.

The histologic structure of the tarsometatarsus resembled that of the humerus. As in the humerus, there was evidence of destruction of old bone and there were numerous Volkmann's canals. There were no white cells in the marrow cavity of either treated or control animals, and there was no indication of the formation of new bone. Within the bony cavities of the shaft, however, small numbers of marrow cells were present. These marrow cells often were in the process of fibrous replacement and thin layers of new bone were found here and there covering the walls of these cavities.

Mallard Drakes

The histologic changes which estrogen produced in the bones of Mallard drakes were in principle the same as those found in Pekin drakes, but were much less extreme. They include increased vasculari-

zation, signs of stasis, marrow fibrosis, appearance of new endosteal bone spicules, and the presence of osteoclasts.

The long bones of control Mallards differed markedly in histologic structure from those of control Pekins. There was much less spongiosa in the Mallard bones, and the number of white cells in the marrow cavity was much lower than in Pekin drakes. As will become clear from our subsequent discussion, the relative scarcity of lymphocytes and pseudo-eosinophils probably had a direct bearing on the lesser response of the Mallard bones to estrogen.

The number of pseudo-eosinophile cells in the marrow was much increased in the animals which had been given the lowest dose of estrogen, but this increase became less conspicuous with increasing amounts of injected hormone. The opposite appeared to be true in regard to the large lymphocytes, *i.e.*, their number was more definitely above normal the more estrogen the animal had received. The multiplication in number of large lymphocytes occurred predominantly along the surface of capillaries where large accumulations of them were found frequently. Some of these cells stained only faintly. There seemed to be transitional stages from these poorly staining lymphocytes to the clear osteoblast-like cells which covered the newly formed bone.

Vascularization of the bones was greatly increased in estrogen-treated Mallards, both the number of capillaries and their lumina being enlarged. In contradistinction to the Pekin drakes, there was little evidence of fibrous degeneration of marrow in the long bones of Mallards, except in those animals which received the highest doses of estrogen.

No evidence of hyperossification was found in the tarsometatarsus. No white cells were observed in the marrow cavity of this bone in either control or treated animals.

There was definite evidence of halisteresis in the tarsometatarsal shaft of treated animals, especially around the haversian canals. The lumina of the haversian canals frequently appeared enlarged. No osteoclasts were present. Bone destruction was observed also in the shaft of other long bones. For instance, there were many Volkmann's canals in the femoral shaft of the Mallard drake which had been treated with the largest amount of estrogen.

Discussion

It can be concluded from our observations that the principal effects of estrogen treatment, as far as bone structure is concerned, are of a similar nature in young and adult cocks and in drakes, although the degree of change varied widely in the different forms which were studied.

For the purposes of the present discussion our interest will center on the nature of the processes which lead to hyperossification. To start with, it is important to emphasize that estrogen treatment may produce extreme degrees of hyperossification in fully grown and sexually mature animals. This was found to be true for Pekin drakes. It is a fact that young cocks respond much more readily and much more extremely to estrogen treatment than do mature cocks. This may be considered an expression of age differences. It is true also, however, that mature Pekin and Mallard drakes, two varieties of the same species, do show even greater diversity with regard to the degree of hyperossification which is produced by excessive doses of estrogen.

It is obvious that age response-differences exist, a fact which has been previously demonstrated for mice by Silberberg and Silberberg.[5] It is clear, too, that strains of the same species may vary widely with regard to the structural bone changes which occur, irrespective of age, after estrogen administration, and this also has previously been reported for mice (Gardner [9]). It appears, therefore, that the skeletal age *per se* and the presence or absence of cartilage in the long bones are not factors which directly regulate or influence the occurrence of hyperossification. Similar conclusions were reached by Sutro and Pomerantz [25] on the basis of observations on estrogen-treated dogs.

It can be seen that definite regularities exist with regard to the extent of estrogen-induced hyperossification if different parts of one and the same bone, or different long bones are compared with each other. In mice, Sutro [14] observed that the lower part of the femur and the upper end of the tibia produced a greater amount of hyperossification than the rest of the appendicular skeleton. Zondek [8] recorded for cockerels that the femur showed more extreme changes than the tibia and the latter much more than the humerus. Pfeiffer and Gardner [21] found that in pigeons the femur exhibited greater alterations than the upper end of the tibia, the latter more than the remainder of the tibia, with still smaller responses for the radius and ulna. Our own findings are of a similar nature. In the leg bones of chickens and ducks the degree of hyperossification was greatest in the femur, less in the tibia and very slight in the tarsometatarsus. The lower end of the femur and the upper end of the tibia underwent more drastic changes than the other parts of these bones. As far as the wing bones are concerned, the situation is less consistent since the humerus, obviously on account of its pneumatic nature, showed little, if any, response to estrogen treatment.

It appears that the order in which the different bones and their parts respond is the product of interaction of a proximodistal gradient (di-

minishing blood supply) and of the order of growth intensity of different parts of the individual bones (probably again a question of blood supply). These sequences are not limited to estrogen treatment, but are found similarly in various pathologic conditions, *e.g.*, Albers-Schönberg (marble bone) disease, rickets, syphilitic osteochondritis (Schmidt [26]), scurvy and osteomyelitis (Harris [27]). The same sequence is evident when bone resorption occurs, as in experimental scurvy, after the administration of phosphorus and lead, and following treatment with parathyroid extract (Jaffe, Bodansky and Blair [28]). This cannot be mere coincidence, but must be an indication of the mechanism by which the phenomena of bone response are put into motion. Since we are dealing with the bones of adult animals, it is likely that the effects of growth intensity are indirect ones, *viz.*, a late consequence of events which occur during growth. An indication of the nature of these events can be seen in the fact that an opposite gradient exists in normal animals with regard to the percentage of ash and calcium in the long bones. In domestic fowls (and also in various mammals), ash and calcium content of the long bones has been found to increase proximodistadly (Harshaw, Fritz and Titus,[28a] Weakley and Dustman,[29] Landauer [30]). The more proximally a bone is located or the higher the growth intensity of one of its parts, the lower is its ultimate calcium content in normal development; and the lower the calcium content of an immature or adult bone, the more calcium will be deposited in response to estrogen treatment.

Our observations indicate that some relationship exists between the amount of endosteal trabeculae which are present in a particular bone and the degree of response elicited by estrogen treatment. This is borne out by comparisons of the behavior of the same bones in different varieties or species, of different bones within the same animal, or even of different parts of one and the same bone. The relationship is not a very close one, however, and the presence of trabecular bone cannot be considered a necessary antecedent to hyperossification. It is more likely that the variations in amount of endosteal bone and the varying degrees of estrogen-induced hyperossification trace back to another factor, common to both. This underlying pattern we believe to exist in the vascular supply to the bones. The observations of von Eggeling,[31] Eckert-Möbius,[32] and others have established the fact that a close parallelism exists between growth intensity of a bone, or of a particular part of a bone, and the degree of its vascularization; in fact, it had already been postulated by Kassowitz [33] that the arrangement of the vascular pattern determines the growth architecture of bone. These persisting morphologic features of earlier growth differences

presumably are responsible, in growing and mature animals, for the specific patterns of bone resorption and of formation of new endosteal bone.

The foregoing interpretation is supported also by the sequence of phenomena as they occur subsequent to estrogen treatment. The first signs which could be discovered in our material always consisted of rarefaction (halisteresis) in the shaft and increased vascularization. The vascular changes, which presumably are responsible for the initial osteoporosis, can be seen in all parts of the bones: shaft, epiphyseal ends and marrow cavity. The haversian canals are widened and increased in number; there are more venules and arterioles. Subsequent vascular phenomena consist of stasis and formation of hematohyaloid. The early vascular changes in the shaft are followed by disappearance of fat from the marrow and by stimulation of marrow cell proliferation. The latter in turn leads to marrow fibrosis, to further vascular changes in the marrow, and finally to formation of endosteal bone.

Many steps in this sequence of alterations are by no means reactions which are exclusive features of bone. It is well known, of course, that estrogen treatment produces vascular effects in the mammalian uterus. Such changes, however, also occur very quickly in other parts of the body, *e.g.*, in the ear of rabbits or in the finger of humans where vasodilation can be observed within a few minutes after estrogen administration (Reynolds and Foster,[34] Reynolds [35]). Formation of hyalin after large doses of estrogen and, secondarily, of epithelioid cells, were observed in the connective tissue of the uterine mucosa and muscularis of mice by Loeb, Suntzeff and Burns[36] and Suntzeff, Babcock and Loeb.[37] These authors stated that transudative changes are among the first found in the uterine wall (see also Arnold, Grumbrecht and Loeser[38]) and that the hyaline substance may be produced by them. Again, it has been shown that in rats estrogen treatment leads to fibrosis in the uterine mucosa and submucosa (Burack, Wolfe and Wright [39]).

These facts indicate clearly that the first skeletal changes, due to estrogen, are closely akin to those produced in other organs. It is only later that development proceeds as a typical local response, by formation of endosteal bone. It should be remembered in this connection that, to use Krompecher's [40] classification, it is only the "primary angiogenic" bone formation which in adult animals is affected by estrogen, and that, as is implied in the terminology, the blood vessels are of particular importance for this type of bone formation. In fact, primary angiogenic bone is the only kind for which it is definitely established that hyperemia leads to formation of new bone substance (Müller [40a]).

It is well known that after fractures of long bones subperiosteal

bone formation occurs frequently at a considerable distance from the location of the fracture (Pollock and Ghormley [41]), and in the case of experimental fractures in canaries Roggemann [42] has described all steps from initial resorption of fatty marrow to spongiosa formation throughout the whole length of the broken and healing bone. These changes also begin with a condition of hyperemia. Fibrosis and sclerosis of the bone marrow, as they occur in many pathologic conditions, can be traced back to hyperemia and blood stasis (Haslhofer [43]). Among these, otosclerosis (otospongiosis) is of particular interest. For, it has been pointed out by Leriche and Policard [44] that this condition is the most characteristic result of vascular disturbance. It occurs most commonly, as the consequence of the calcium drainage during pregnancy, in women with great vasomotor sensibility of the face. It begins with resorption phenomena (rarefaction of bone) and ends in bone condensation (sclerosis).

Many details of the estrogen-induced bone changes bear a definite resemblance to the osteosclerotic phenomena which occur in certain blood diseases (pseudoleukemia, hemorrhagic aleukemia, polycythemia) and the sequence of changes appears to be much the same in the two instances (Schmidt,[26] Wolf,[45] Mettier and Rusk [46]). There is reason to believe that in many, if not all, cases of this nature the bone changes are primary (Jordan and Scott [46a]), and it may well be that the initial steps which lead to these osseous abnormalities are similar to those produced by estrogen.

It has been shown that hyperossification exists as a part of a seasonal rhythm in the females of certain species of birds. This is true of pigeons (Kyes and Potter,[47] Bloom, Bloom and McLean [48]), English sparrows (Kirschbaum, Pfeiffer, van Heuverswyn and Gardner [22]), quail (Ringeon [49]), chickens (Bloom and Domm [50]) and ducks (Bloom, Bloom, Domm and McLean,[19] Benoit, Grangaud and Sarfati [51]). It may be assumed that the naturally occurring hyperossification and that produced by estrogen administration are both brought about by similar vascular stimuli. Estrogen appears to produce vasodilation in those parts which are either prominent heat-losing surfaces (ears, fingers) or which, for physiologic reasons, are especially responsive (uterus of mammals, marrow bones of birds). The rhythmic process of bony hyperossification in birds is an obvious adaptation to reproductive phenomena (shell formation) and as such occurs only in females. It appears from our observations, however, that the readiness of the bone circulation to respond to estrogen is present in males as well. Large doses of estrogen evidently produce vascular over-stimulation, leading to other pathologic processes (arteriolar hypertension, aneurysm).

All of our observations on endosteal bone formation, as a consequence of estrogen treatment, are, in our opinion, but a confirmation and extension of the statement by Leriche and Policard [44] (page 32) that:

"Toutes les modifications dont nous venons de parler: oedème, multiplication des fibrilles, condensations préosseuses, paraissent dépendre des variations du régime circulatoire. Sans elles, ces modifications ne se produisent pas. Quand elles ont lieu, le régime circulatoire est toujours modifié. En définitive, la création d'un milieu ossifiable et la condensation préosseuse paraissent n'être que la conséquence des modifications circulatoires."

Our observations fully agree with and give weight to Leriche's [52] (p. 310) generalization that:

"Il en résulte immédiatement ceci que, dans toutes les maladies avec ossifications, petites ou grandes, *l'ossification est, pathologiquement parlant, ce qu'il y a de moins important à étudier*. . . . . Pour trouver le pourquois des choses, il faut remonter à l'origine, chercher la cause et le mécanisme de la *raréfaction* qui a tout précédé, parce que c'est là que se trouve la raison immédiate de la néoformation osseuse. A l'origine de toute ossification, il y a un *processus de vaso-dilatation active* . . . ."

The early vascular changes can only lead to the formation of endosteal bone if the proper conditions are present within the marrow or bone cavities. Fibrous degeneration of marrow and hyalin furnish the substratum for calcification and ossification. In the absence of red marrow and blood vessels the processes leading to endosteal bone formation cannot occur. This situation presumably explains the differences in degree of response to estrogen treatment which exist between Mallard and Pekin drakes. The long bones (femur, tibia) of the latter are rich in red marrow and well vascularized, while those of Mallards have few white cells in their marrow cavities and a relatively poor blood supply. Similar conditions probably account for the differences in response between the tarsometatarsus and the other long bones of the leg.

It was found that some of the earliest skeletal symptoms of estrogen treatment (increased vascularization, stasis, formation of hyalin) can be produced likewise by injection of cholesterol (Fig. 21). No lipemia was observed in these cholesterol-treated animals (Zondek and Marx [53]). It is unlikely, therefore, that the lipemia which occurred as a consequence of estrogen injections was responsible for the circulatory changes which appear to mark the beginning of bone responses to estrogen.

The injection of oil (olive oil) alone produced a slight increase in vascularization of the bone marrow. All of the changes in bone which follow the injection of estradiol benzoate can, however, be called forth by the implantation of pellets of the hormone. Hence, it is clear that

the solvent (sesame oil) which was used in our experiments did not play a rôle in initiating the skeletal abnormalities.

Our material in no way suggests that estrogen has a specific stimulating effect on multiplication or activity of osteoblasts. On the contrary, all observations point to the conclusion that the eventual formation of large amounts of endosteal bone after estrogen treatment results chiefly, if not entirely, from metaplasia of the myeloid elements. This metaplasia may well be of the nature of a temporary cicatrization of the degenerating marrow.

It should be emphasized that the vascular stimulation in the bones of cholesterol-treated animals was not accompanied or followed by the appearance of new bone, whereas such bone formation did occur after estrogen injections. We are inclined to believe that these differences are of a quantitative nature, estrogen producing a more drastic vascular stimulation, but the existence of still other and more specific effects of estrogen is not excluded by the present evidence.

Finally, it should be pointed out that there was no obvious correlation between the size of the testes of the estrogen-treated animals and histologic structure of the bones. This holds for domestic fowls and both kinds of ducks. In fact, no consistent difference could be seen between cocks and capons in the kind and degree of effect which estrogen had on the long bones.

Summary

Observations on long bones of cocks and drakes, which had been treated with estradiol benzoate, led to the following conclusions:

1. The earliest changes consist in an increased vascularization of the shaft of the long bones, accompanied by removal of bone salts (osteolysis).

2. The processes of halisteresis are followed by disappearance of fat from the marrow cavity, increased multiplication of marrow cells and vasodilation in the marrow.

3. After large doses of estrogen the marrow undergoes fibrous degeneration with focal necrosis, production of hyalin within and outside of blood vessels, and eventually the formation of new endosteal bone. Thickening of the walls of small vessels occurred at the same time.

4. There is a definite pattern of response of the various long bones. In the leg the femur shows the greatest changes, the tarsometatarsus the least. Similar patterns of response can be observed with regard to different regions of one and the same bone. These patterns correspond closely to the known differentials of growth activity and blood supply.

5. The skeletal changes after estrogen treatment are slight in Mal-

lard drakes and very pronounced in Pekin drakes, with cocks holding an intermediate position. Young cockerels show a much greater response than mature cocks. The effects are similar in mature cocks and fully grown capons.

6. It is believed that circulatory adjustments initiate the sequence of events which leads to final hyperossification, and that the formation of new endosteal bone represents a cicatrization of the degenerating marrow.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 32

FIG. 1. Roentgenogram of femur and tibia of cockerel H75. Age, 11 weeks. Treatment: 5 mg. of estradiol benzoate twice weekly for 5 weeks. Bone density increased.

FIG. 2. Roentgenogram of femur and tibia of cockerel H62. Age, 18 weeks. Treatment: 5 mg. of estradiol benzoate three times weekly for 12 weeks. Resorption of old and deposition of new bone.

FIG. 3. Roentgenogram of femur, tibia and tarsometatarsus of cockerel H29. Age, 42 weeks. Treatment: 2.5 mg. of estradiol benzoate three times weekly for 36 weeks. Extreme bone condensation in femur and upper part of tibia; slight changes in lower end of tibia; normal tarsometatarsus.

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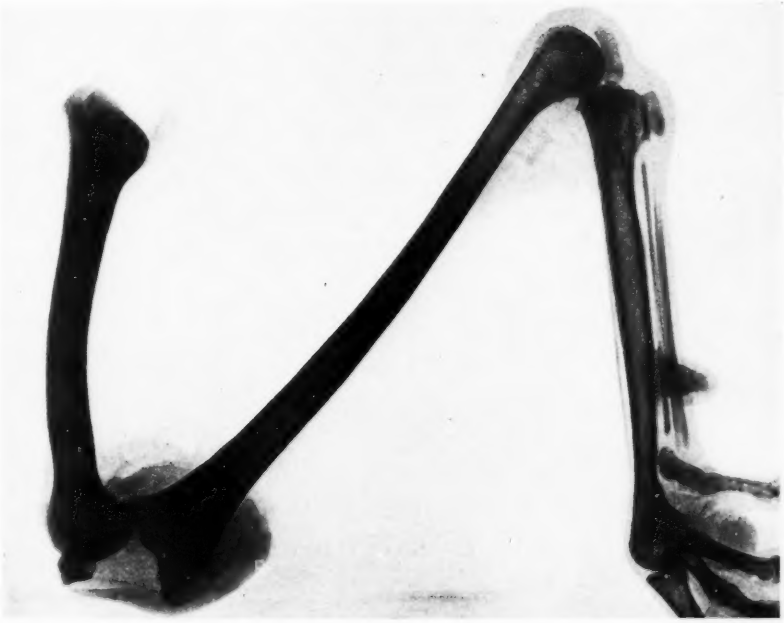


3

Landauer and Zondek Bone in Estrogen-Treated Birds

PLATE 33

FIG. 4. Roentgenograms of femora of adult Pekin drakes. From top to bottom, P_2, P_1, P_4, P_5, P_3. The first and last bones (P_2 and P_3) are from untreated controls. The other bones are from animals which had been treated for 3 weeks with, respectively, 0.8 (P_1), 1.0 (P_4) and 2.0 (P_5) mg. of estradiol benzoate daily.

4

Landauer and Zondek Bone in Estrogen-Treated Birds

PLATE 34

FIG. 5. Roentgenograms of tibiae of the same Pekin drakes as shown in Figure 4. The changes in the tibia are less in degree than those in the femur. There is irregularity in bone deposition, and new bone is absent in the lower end of the tibia.

Landauer and Zondek Bone in Estrogen-Treated Birds

PLATE 35

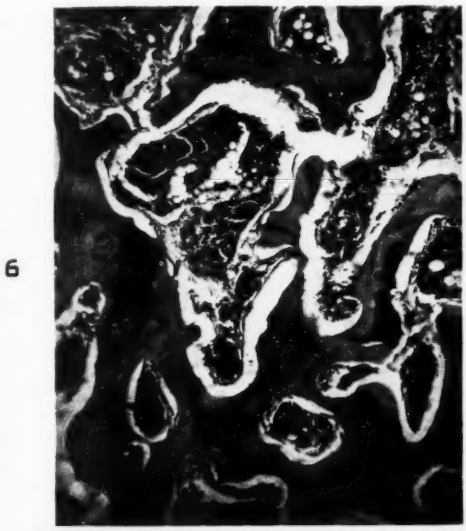
FIG. 6. Cock 5646, injected with 0.17 mg. of estradiol benzoate daily for 26 days. Frontal section of femoral metaphysis, showing halisteresis of old spongiosa. × 150.

FIG. 7. Cock 4670, injected at first with 0.23 mg. of estradiol benzoate daily; dose later increased to 0.6 mg. Treated for 61 days. Total dose, 25.4 mg. Cross section of femoral diaphysis. To the right and below, shaft with osseous cavity containing new bone. To the left, newly formed bone in marrow cavity. Fibrous transformation of marrow. × 150.

FIG. 8. Cock 5646. Frontal section of tarsometatarsal shaft. Fibrous degeneration of marrow in osseous cavities of periosteal bone. × 45.

FIG. 9. Cockerel H29. Complete cross section of femoral shaft. Large numbers of new endosteal bone trabeculae are seen. Compare with Figure 15. × 6.5.

FIG. 10. Cockerel H75. Cross section of tibial shaft. New endosteal bone. Three vessels with thickened walls. Large masses of pseudo-eosinophils which have undergone some degree of fibrous degeneration. × 45.



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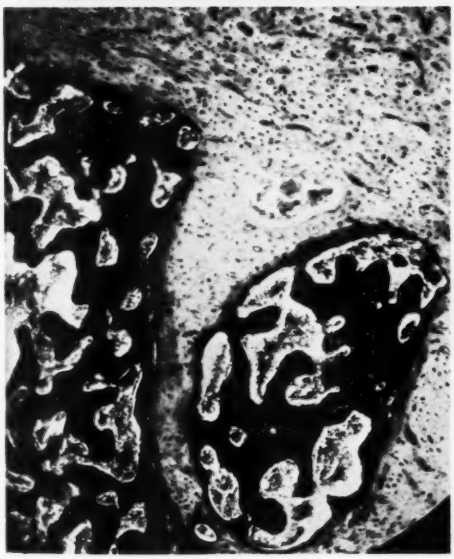

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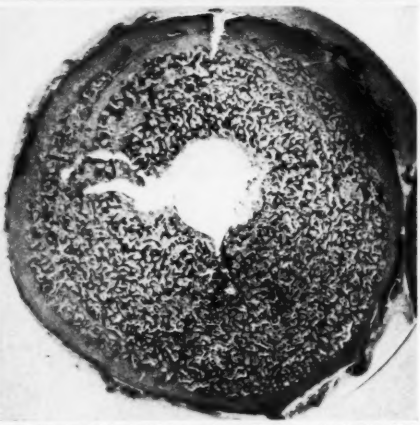

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10

Landauer and Zondek Bone in Estrogen-Treated Birds

Plate 36

Fig. 11. Cockerel H_{59}, treated three times weekly for 3 weeks with 5 mg. of estradiol benzoate. Killed when 9 weeks old. Cross section of tibial diaphysis, showing a much enlarged venule filled with hyaline material. × 70.

Fig. 12. Frontal section through distal femoral metaphysis of Pekin drake P_4. Old (above and to left of center) and new endosteal bone. Hyperplastic marrow and extensive marrow fibrosis. Hyalin in upper left corner. × 45.

Fig. 13. Frontal section through proximal tibial metaphysis of Pekin drake P_5. Red cells and hyalin between trabeculae of new endosteal bone. × 155.

Fig. 14. Cross section of fibular side of tibial diaphysis of Pekin drake P_1. The inner surface of the large osseous cavity of the shaft, located here, is the only place where new bone was present. × 15.

Fig. 15. Complete cross section through the femoral diaphysis of Pekin drake P_5. Compare with Figure 9. Large amounts of new endosteal bone are present. × 6.5.

Fig. 16. Frontal section through the proximal tibial metaphysis of Pekin drake P_1. New bone trabeculae and osteoclasts. × 155.

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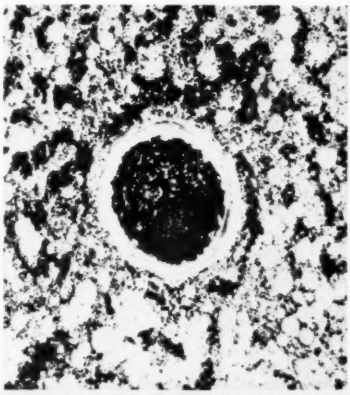

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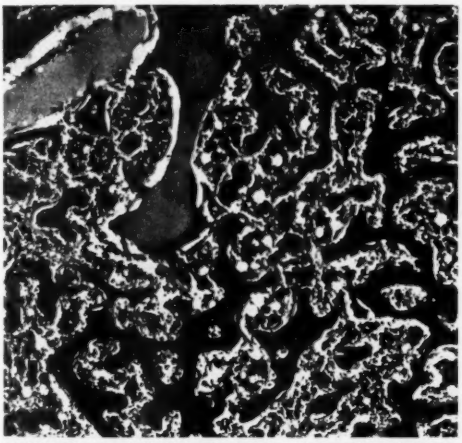

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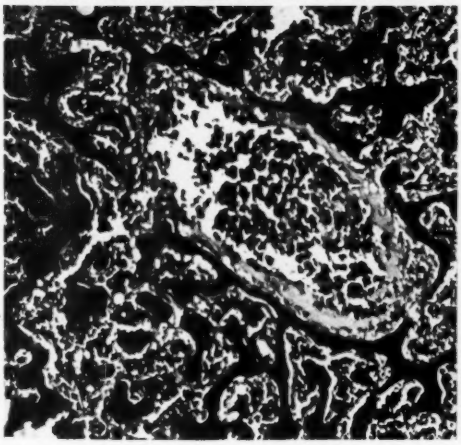

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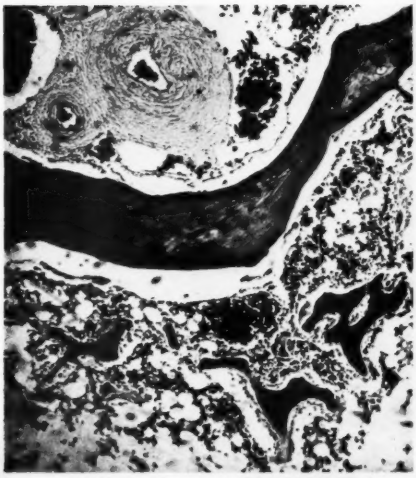

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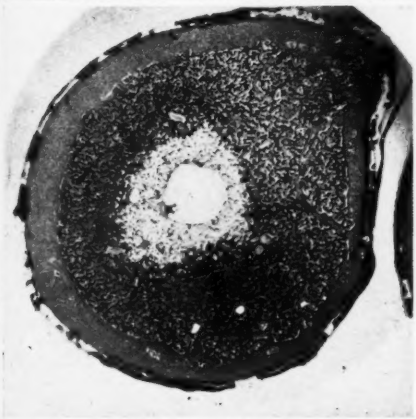

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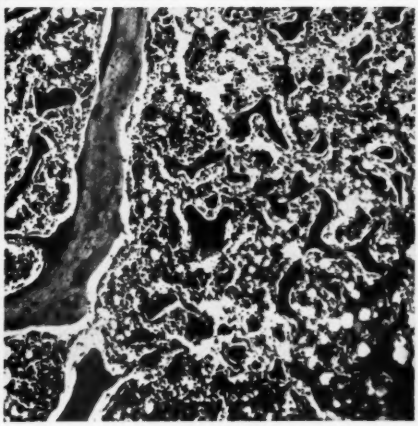

PLATE 37

FIG. 17. Frontal section through the proximal tibial metaphysis of Pekin drake P_5. Cartilage in old endosteal bone, new bone spicules, complete fibrosis of marrow. × 55.

FIG. 18. Frontal section through the proximal tibial metaphysis of Pekin drake P_4. Many new endosteal trabeculae, hyperplastic marrow, hyalin outside of vessels. × 45.

FIG. 19. Frontal section through the proximal tibial metaphysis of Pekin drake P_4. Venule with hypertrophied wall and hyalin in lumen, surrounded by new bone. × 150.

FIG. 20. Cross section of the humeral diaphysis of Pekin drake P_5. Much enlarged haversian canals in the shaft. × 150.

FIG. 21. Cross section of the femoral shaft of cockerel H74, treated with cholesterol (twice weekly 5 mg. of cholesterol in olive oil for 2½ weeks, *i.e.*, a total of 25 mg.). Age, 8½ weeks. Great increase in the number and widening of the haversian canals. × 45.

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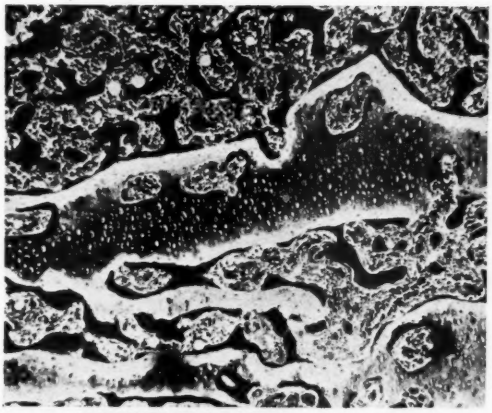

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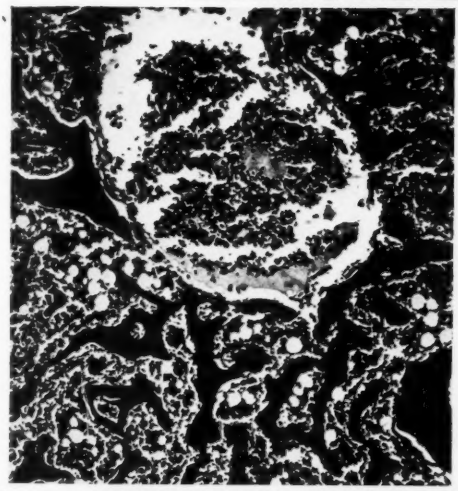

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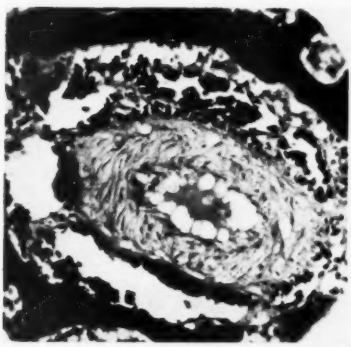

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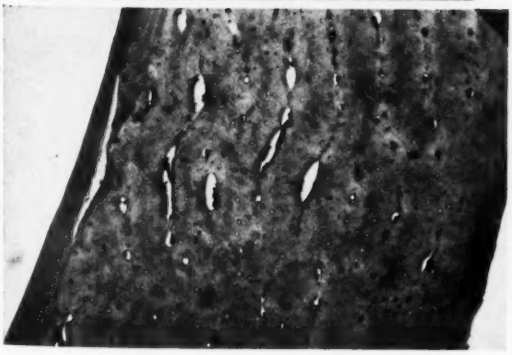

21

Landauer and Zondek

Bone in Estrogen-Treated Birds

EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

VIII. Late Vascular Reactions of Histamine Shock in Dogs *

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In a previous publication [1] mention was made of the lesions noted in the organs and vessels of dogs which died within 48 hours after a histamine shock elicited by the subcutaneous injection of a suspension of this substance in a mixture of cotton seed oil and "Falba" absorption base.† It was noted then that an appreciable percentage of these dogs revealed marked edema and necrosis of the wall of the gallbladder, as well as hemorrhagic erosions and single or multiple ulcers in the prepyloric region of the stomach, with dilated and thrombosed vessels and arterioles with swollen and hyaline walls in the submucosa. There were also hemorrhages and leukocytic and mononuclear infiltrations with myocardial degeneration in the heart, associated with arterioles with swollen walls. In addition there was congestion and hemorrhage in the liver, and in the duodenal and jejunal mucosa, and hemorrhagic infarct-like areas in the spleen.

In subsequent experiments in which the same method of shocking was used, the organs of dogs which survived the shock and which were killed 8 days after this episode were studied. An opportunity was thereby provided to investigate the late effects in the various organs resulting from such circulatory and metabolic trauma. The present communication contains the results of this study.

Experimental Procedure

A detailed histological investigation was made of the organs of 5 dogs weighing between 8 and 15 Kg. and belonging to a group of 100 dogs used to study the efficacy of methyl cellulose-S-methyl isothiourea solution in the treatment of histamine shock.[2] These particular dogs, among others, had survived the shock episode for 8 days. The shock had been elicited by the subcutaneous injection of histamine dihydrochloride (15 mg. per Kg. of body weight) suspended in a mixture of cotton seed oil (U.S.P.) and "Falba" absorption base (3 parts of oil to 1 part of absorption base). One cc. of the vehicle contained 20 mg. of histamine dihydrochloride. After such an injection the blood pressure dropped from a level of above 100 mm. Hg to 30 to 40 mm. within

* Received for publication, April 6, 1943.

† Falba is an oxycholesterin absorption ointment base, manufactured by Pfaltz and Bauer, Inc., New York, N.Y.

10 to 20 minutes and remained at approximately this level for several hours unless the treatment was instituted. Femoral blood pressure of dogs, which had been given nembutal, was recorded on a kymograph by means of a mercury manometer. The treatment, given 1 to 2 hours after the blood pressure had become stationary at the stated low level, consisted of intravenous infusions of a 0.075 per cent methyl cellulose (4000 centipoises) solution in normal saline to which S-methyl isothiourea (1:1000) had been added. Between 200 and 350 cc. of this agent, representing a combination of an aqueous solution of a highly viscous and hydrophilic colloid with a vasotonic substance, were given to the individual dogs. The blood pressure was restored thereby to, or near, the original level. After a preliminary observation period of 7 hours the dogs were removed from the operating table following ligation of the femoral artery, dusting of the wound with sulfathiazole and suturing of the skin. Eight days later they were sacrificed by the intravenous injection of 20 cc. of a 4 per cent formaldehyde solution. The autopsy was performed immediately afterward.

In general the gross examination revealed normal organs. One dog showed a stomach ulcer which was located about 1 inch above the pylorus and which measured approximately 1 cm. in diameter. The shallow floor of the ulcer consisted of a pale whitish tissue.

Histological Study

Brain. Minor small, round, cellular accumulations were present around the vessels in the region of the Ammon's horn in one dog. The vessels in the region of the basal ganglia of a second dog appeared as dark blue-stained tubes, while the meninges contained a mononuclear infiltration.

Hypophysis. The anterior lobe consisted mainly of eosinophilic cells, which, in one case, surrounded a small, basophilic, cellular accumulation. The posterior lobe was congested.

Lung. There were some scattered edematous areas present in the lungs of four dogs. The lung of the fifth dog was normal.

Heart. The myocardium, particularly the septum and papillary muscles, showed foci of mononuclear cells and fibroblastic proliferations, involving areas of granular muscular degeneration. Calcification of single muscle cells or groups of muscle cells was found in two dogs (Fig. 1). These degenerative changes were relatively extensive in one dog. The myocardial arterioles exhibited in one dog hyaline, polypous, intimal thickenings. In a second dog the arteriolar walls were occasionally swollen, hyaline and infiltrated by a few mononuclear cells.

Aorta. The middle and outer media of one aorta contained foci of

hyalinization and fibroblastic proliferation engulfing individual muscle bundles, while the vasa vasorum in the adventitia were surrounded by hyaline sheaths (Fig. 2). The middle section of the media in a second dog revealed a small focus of liquefaction necrosis which was surrounded by an increased number of muscle cell nuclei in radiating arrangement (Fig. 3). Small, bluish, calcified areas were present beneath the intima of the ascending and descending parts in a third dog, representing apparently calcified imbibition fluid in the subintimal space. A superficial diffuse calcification of a hyaline intimal thickening was found in one section, while in the second section a large fibrohyaline area existed in the middle and outer media (Fig. 4). The carotid artery contained also several subendothelial, granular calcium deposits. The middle media of the fourth dog revealed small foci of fibroblastic proliferation and a considerable edema of the inner media in the region of the arch. Beneath a diffuse intimal thickening, consisting of circularly arranged fibroblastic cells, small cavities were present bordering toward the outside on the internal elastic membrane (Fig. 5). The inner media was edematous, beneath a mucohyaline intimal thickening located 1 inch above the aortic valve (Fig. 6). Sections stained for elastic fibrils showed that the cavities were free from these elements and that the elastic fibrils in the adjacent parts of the aortic wall were rarefied and fragmented, while they were densely packed in some of the hyalinized areas of the media. The other large elastic arteries (carotid, femoral) were normal in all dogs with the exception of the one carotid artery mentioned.

Liver. There were numerous pericentral necroses present, associated either with congested capillaries or edema, or with fibroblastic proliferation containing numerous capillaries which gave to these foci an angioma-like appearance. The latter lesion was accompanied by an occasional hyaline thickening of the arteriolar walls. The Kupffer cells were swollen and loaded with a dirty greenish gray pigment. Similar cells were found within the fibroblastic pericentral foci.

Gallbladder, Pancreas, Intestine, Adrenal. Normal.

Stomach. The mucosa exhibited in one dog a defect which was filled by a proliferating fibroblastic mass which contained in its superficial parts hyalinized matter and leukocytes. The edematous submucosa showed dilated and hyperemic vessels. Some of the arteries in the submucosa had swollen hyaline walls. The stomachs of the other four dogs were normal.

Spleen. The pulp of one dog presented areas consisting of a fibroblastic thickened network, the meshes of which were empty. A coarse angiectatic structure was thus produced. The spleens of the other dogs

were normal except for some increase in brown-yellow hematogenous pigment.

Kidney. The tubular epithelium was vacuolated and frayed. There were scattered mononuclear foci in the interstitial tissue.

Comment

The late vascular and related organic lesions observed in dogs subjected to a fatal histamine shock illustrate again that a hypotensive mechanism can be just as effective in the production of vascular degenerative and sclerotic changes as a hypertensive mechanism.[3, 4] These observations support the concept of Lange,[5] who contended that arteriosclerosis is the result of a paresis of the innervated muscular media and a sequela of arteriohypotonia which causes a stagnation of tissue fluid in the vascular walls. They corroborate and extend those observations previously made by Heinlein,[6] Meessen [7] and Rühl,[8] who found in rabbits chronically poisoned with histamine that the pulmonary arteries had a swollen and loosened media and local intimal thickenings, that the media of the coronary arteries was replaced by connective tissue and that the myocardium contained numerous necroses and hemorrhages. Meessen as well as Heinlein attributed these changes to the production of a stagnant anoxemia resulting from circulatory failure. The ensuing endothelial damage and increased vascular permeability to plasma ultimately leads to the development of sclerotic lesions.

While the dogs previously studied which died after a short interval of histamine poisoning exhibited the early lesions characteristic of circulatory disturbances (stasis; hyaline and erythrocytic thrombosis) and vascular hypotonic permeability (swelling, edema and hyalinization of walls), those of the present investigation reveal definite late effects of such episodes upon the vascular wall. The bluish-stained incrustations of the cerebral vessels of one dog are anatomically and topographically similar to those found as the result of anoxemic episodes elicited by carbon monoxide and lead poisoning. Morphologically they carry a close resemblance to the pseudocalcifications of the cerebral vessels described by Ostertag,[9] who related their massive appearance to endocrinic disturbances of the calcium metabolism.

Although the occurrence of calcification of the subintimal plasmatic fluid and of the muscle cells of the heart, present in several dogs, might support this view, such changes may occur also in the absence of demonstrable disorders of calcium metabolism when necrosis occurs in the myocardium and vascular walls (adrenalin necroses). The repeated observation of small cavities in the aortic media, representing the re-

sults of liquefaction necrosis preceded apparently by a local loss of nuclei and homogenization of the media and followed by the formation of a fibroblastic scar, has a certain similarity to the medionecrosis aortae idiopathica cystica described by Erdheim.[10]

The therapeutic agents administered in an attempt to control an otherwise fatal shock have no part in the production of the described vascular and organic changes, as the intravenous injection of methyl cellulose solution in amounts far exceeding those given to the dogs in this experiment elicits lesions which differ fundamentally from those reported here.

Conclusions

Dogs surviving a severe, prolonged and usually fatal histamine shock developed degenerative, cystic and calcifying lesions in the aorta, particularly of its media, as well as in the smaller arteries (carotid, coronary, cerebral, gastric). The heart showed myocardial degenerations, with calcification and mononuclear and fibroblastic foci.

The most striking late results of the transitory circulatory and vascular reactions of histamine shock were: prepyloric peptic ulcers, pericentral hepatic necroses with hemorrhages and fibroses, and focal splenic fibroblastic proliferations.

The observations provide additional experimental evidence for the claim that hypotensive agents may elicit sclerotic arterial lesions.

The late vascular, cardiac and parenchymatous organic lesions observed after acute but prolonged histamine shock may thus not only provide the anatomical basis for delayed deaths after shock, but also for cardiovascular changes attributed in general to arteriosclerosis.

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DESCRIPTION OF PLATES

PLATE 38

FIG. 1. Mononuclear interstitial infiltration of the myocardium with granular calcification of individual muscle cells. Hematoxylin and eosin stain. × 230.

FIG. 2. Homogenization of parts of the outer media of the aorta with hyaline sheaths around the vasa vasorum in the adventitia. Hematoxylin and eosin stain. × 120.

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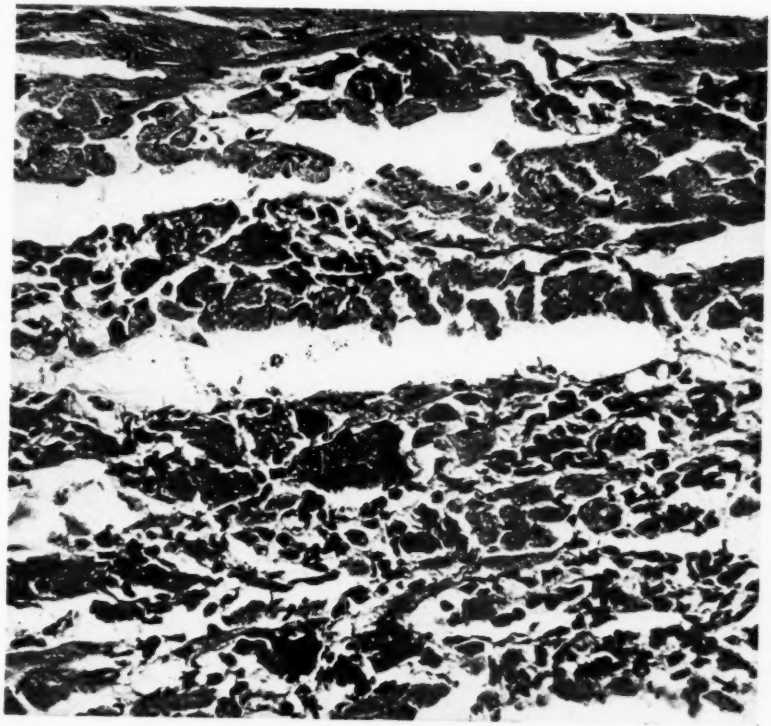


2

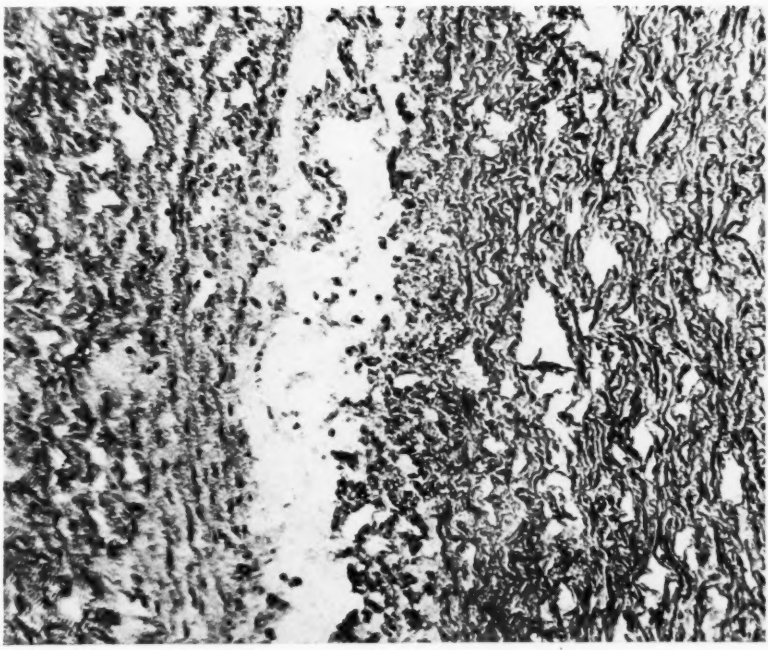
Hueper and Ichniowski Vascular Reactions of Histamine Shock

PLATE 39

FIG. 3. Cystic cavity with ragged edges containing a faintly stained and scanty albuminous material located in middle third of media of the aorta. Hematoxylin and eosin stain. × 120.

FIG. 4. Large area of mucinous hydropic degeneration surrounding vasa vasorum with thickened walls in the aortic media. Hematoxylin and eosin stain. × 120.

3



4

Hueper and Ichniowski Vascular Reactions of Histamine Shock

PLATE 40

FIG. 5. Two large cystic cavities with degenerative changes in the adjacent tissue are situated in the middle media of the aorta. A large area of homogenization is located in the outer third of the media Hematoxylin and eosin stain. × 120.

FIG. 6. Much thickened aortic intima with small cystic cavity filled with a mucinoid material located adjacent to internal elastic membrane. Hematoxylin and eosin stain. × 150.

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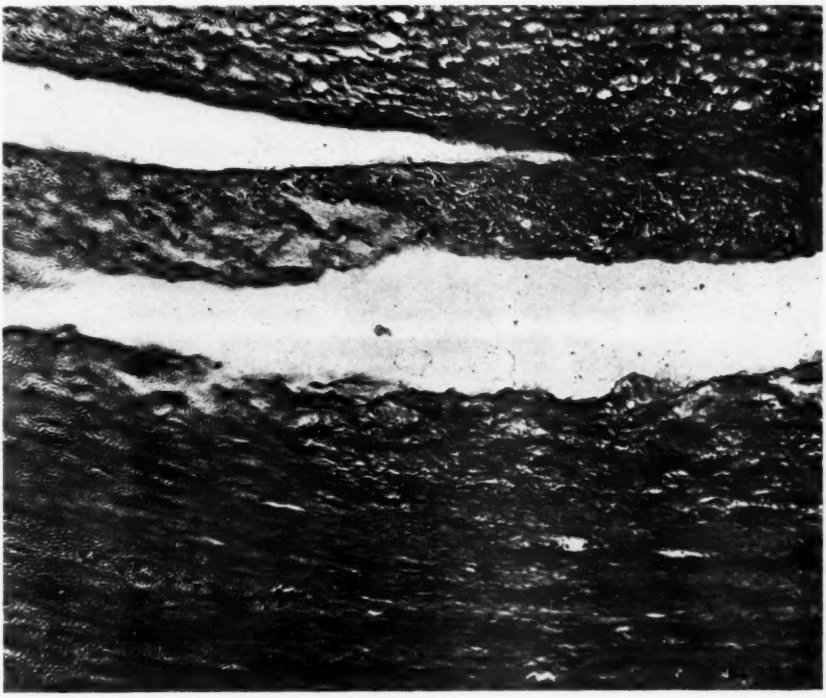

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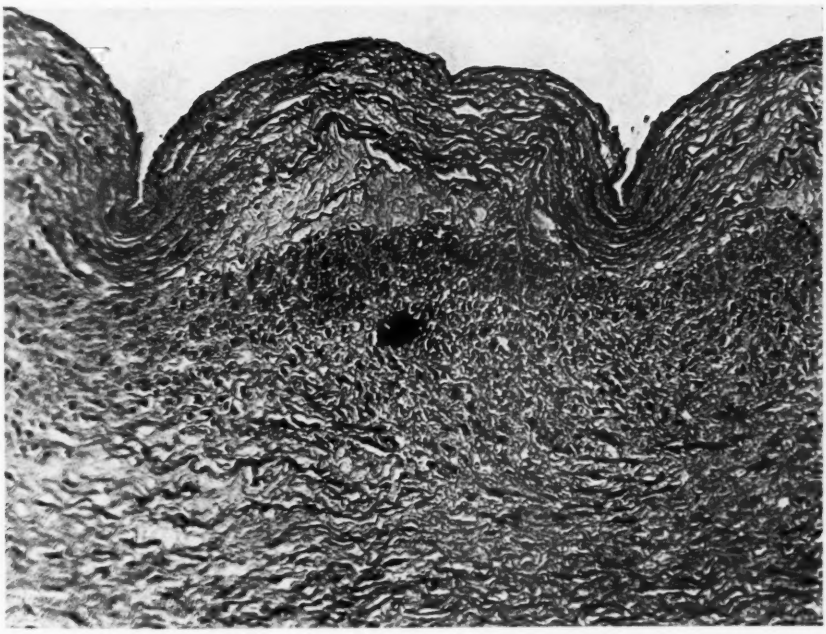

Hueper and Ichniowski Vascular Reactions of Histamine Shock